



## **The quality of cold smoked salmon**

### **Influence of raw material and technological parameters**

**Løje, Hanne**

*Publication date:*  
2007

[Link back to DTU Orbit](#)

*Citation (APA):*  
Løje, H. (2007). *The quality of cold smoked salmon: Influence of raw material and technological parameters.*

---

#### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# THE QUALITY OF COLD SMOKED SALMON

- Influence of raw material  
and technological parameters

Hanne Løje  
Ph.D. thesis

Technical University of Denmark

Danish Institute for Fisheries Research  
Department of Seafood Research

BioCentrum

2007



---

**The quality of cold smoked salmon**  
**- Influence of raw material and technological parameters**

Hanne Løje

Ph. D. thesis

Technical University of Denmark

Danish Institute for Fisheries Research

Department of Seafood Research

BioCentrum

2007

---

## PREFACE

This Ph. D. thesis is submitted as a requirement for obtaining the Ph. D. degree at the Technical University of Denmark (DTU). The Ph. D. project was carried out at the Danish Institute for Fisheries Research, Department of Seafood Research (DIFRES) in Lyngby during the period June 2003-May 2007. The work was financially supported by a grant from The Danish Ministry of Food, Agriculture and Fisheries.

First of all, I would like to thank my supervisors Jette Nielsen, Grethe Hyldig and Henrik H. Nielsen for their support, constructive advice and guidance during this study. Also thank for their constructive comments on the manuscripts.

My colleagues at DIFRES, especially at the Raw material, Process and Product group are thanked for creating a good working environment. Bo Jørgensen is thanked for encouraging scientific discussions and critical comments on the manuscripts. Thanks to Kristina N. Jensen for many interesting discussions and valuable knowledge in the area of multivariate data analysis. Hugo Ladefoged, Carsten Østerberg, Ulrik Cold, Inge Holmberg, Rie Sørensen, Jeannette U. Møller, Claus Reesbøll, Karin Reimers and Susan S. Jensen are thanked for their invaluable technical assistance in the laboratory. I would like to thank Søren T. Christensen for providing me with literature and Sally Clink for proofreading of this manuscript. Maria Randrup is thanked for reviewing my thesis.

The involved cold smoked salmon industries are appreciated for preparing the experimental material. Thanks to Højmarklaboratoriet and Biomar for a fruitful cooperation. Dagmar Bruggemann and Moira Lawson, at the Meat Department at the Faculty of Life Science at Copenhagen University are thanked for assistance with the confocal microscopic analysis. Thanks to Monika Victor-Hansen for making the front page and to Hugo Ladefoged and Erik Lund for the pictures.

Also thanks to my family and friends for help and encouragement especially during the last months of intensive writing. A special thank, to my husband Henrik for his patience and support and to my daughter Caroline – thank you for your smile and happiness.

Lyngby, May 2007

Hanne Løje



---

## SUMMMARY

The objective of this Ph. D. thesis was to study the liquid holding capacity/liquid loss of raw and smoked salmonids as affected by raw material and chill storage of the cold smoked product.

The liquid holding capacity is an important quality parameter for cold smoked salmon. This study has shown that the liquid holding capacity in raw and cold smoked salmon is influenced by several factors. The size of the fish affected the liquid holding capacity as large fish had lower liquid holding capacity than smaller fish. The salt content influenced the liquid holding capacity in smoked fish as it was found that high salt content gave higher liquid holding capacity. The salt uptake of the fillets was affected by the lipid content as a high lipid content lead to a lower salt content. It was also found that the lipid content increased with the size of the fish. The lipid content affected the liquid holding capacity in raw salmon, as high lipid content gave lower liquid holding capacity. Thus, the lipid content is an important parameter regarding the liquid holding capacity as it can influence the liquid holding capacity directly or indirectly by affecting other factors e.g. the salt content which influences the liquid holding capacity.

During the chill storage period of smoked salmon, the liquid holding capacity decreased. It was found that the large smoked salmon lost more liquid than the small smoked salmon did during chill storage. At the same time the lipid fraction of the liquid loss increased while the water fraction remained at a constant level. The decrease in the liquid holding capacity during chill storage of the smoked product was related to changes in the water distribution. Three water pools were found in raw and smoked salmon samples. An exchange of water from pool II to pool I was seen during chill storage of smoked salmon. The microstructure of the fish muscle was affected by the smoking process and the subsequent chill storage. An indication of lipid droplets being released was observed during the chill storage period, which could indicate denaturation of the collagen structure in the muscle.

Several methods for measuring the liquid holding capacity in fatty fish have been used. A comparison of two of the methods, a centrifugation method and a liquid leakage test, was made. The investigation showed that the two methods measure different parameters, and

---

that the two methods cannot substitute each other and the methods may have different applications. Both methods are highly dependent on experimental conditions. It is recommended that both types of methods are used in order to get a detailed picture as possible of the liquid holding capacity. NMR relaxation curves were used to investigate the relation between the centrifugation method and the liquid leakage test. A high correlation was found between NMR relaxation curves and the liquid holding capacity measured by the centrifugation method for both rainbow trout and salmon. Thus, the low-field NMR technique has potential as a fast and non-destructive method to measure liquid holding capacity in fatty fish.

In conclusion, this study has shown that the ability of the salmonid muscle to hold liquid is a complex property influenced by many factors. The experiments in this thesis have shown that raw material and chill storage of the smoked product affected the liquid holding capacity. Thus, the producers of cold smoked salmon should be aware of this and should have a careful control of the raw material especially regarding the lipid content.

---

## SAMMENDRAG (DANISH SUMMARY)

Formålet med dette Ph. D. projekt var at undersøge om væskebindingsevnen/væsketab i rå og røget laks var påvirket af råmateriale og efterfølgende kølelagring af det koldrøgede produkt.

Væskebindingsevnen er en vigtig kvalitets parameter for kold røget laks. Dette studie har vist, at væskebindingsevnen i rå og koldrøget laks er påvirket af mange parametre. Størrelsen af fisken påvirkede væskebindingsevnen, idet store laks havde mindre væskebindingsevne end små laks. Saltindholdet påvirkede væskebindingsevnen i røgede fisk, idet højere salt indhold gav bedre væskebindingsevne. Salt optaget var påvirket af fedtindholdet, da et højt fedtindhold førte til et lavere salt indhold. Fedtindholdet steg med fiskens størrelse. Fedtindholdet påvirkede væskebindingsevnen i rå laks, idet et højere fedtindhold gav lavere væskebindingsevne. Fedtindholdet er en vigtig parameter, da det kan påvirke væskebindingsevnen direkte eller indirekte ved at påvirke andre faktorer som f.eks. salt og derved påvirke væskebindingsevnen.

Under kølelagring af røgede laks, sås et fald i væskebindingsevnen. Store røget laks tabte mere væske end små røget laks i løbet af kølelagringsperioden. På samme tid blev der set en stigning i fedtdelen af væsketabet, mens vanddelen i væsketabet forblev på et konstant niveau. Faldet i væskebindingsevnen i løbet af kølelagringen af det røgede produkt var sammenfaldende med ændringer i vandfordelingen. Der blev fundet tre vand pools i rå og røgede laks. Under kølelagringen af røgede laks skete der en udveksling af vand mellem vandpool I og vand pool II. Der skete ændringer i mikrostrukturen, som følge af koldrøgningsprocessen og som følge af kølelagringen. Der blev set en indikation af frigørelse af fedtdråber i løbet af kølelagringsperioden, hvilket kunne indikere nedbrydning af collagen strukturen i musklen.

Forskellige metoder har været anvendt til at bestemme væskebindingsevnen i fede fisk. Der blev lavet en sammenligning af en centrifuge metode og en væskeslips metode. Undersøgelsen viste, at disse to metoder måler forskellige parametre, og at de ikke kan erstatte hinanden, men de kan have forskellige anvendelsesmuligheder. Endvidere er begge metoder meget afhængig af de fysiske forsøgsomstændigheder. Det anbefales at anvende

---

begge metoder for at få et nuancerede billede som muligt af væskebindingsevnen. NMR relaxations kurver blev anvendt til at undersøge sammenhængen mellem de to ovennævnte metoder. Der blev fundet en høj korrelation mellem centrifuge metodens resultater og NMR målingerne for både regnbueørreder og laks. Low-field NMR forventes derfor at have et potentiale som en hurtig og ikke-destruktiv metode til bestemmelse af væskebindingsevnen i fede fisk.

Som konklusion, så har dette studie vist at lakse musklens evne til at holde væske er en kompleks parameter, der kan påvirkes af mange faktorer. Forsøgene i denne afhandling har vist, at både råvaren og efterfølgende kølelagring påvirkede væskebindingsevnen. Det er derfor vigtigt for producenter af koldrøget laks at være opmærksom på dette, og stille krav til deres råvare specielt med hensyn til fedtindhold.

---

## ABBREVIATIONS

CLSM	Confocal laser scanning microscope
CPMG	Carr-Purcell-Meiboom-Gill
CV	Cross validation
DIFRES	Danish Institute for Fisheries Research, Department of Seafood Research
DHA	Docosahexaenoic acid
DKK	Danish kroner
DM	Dry matter
DTU	Technical University of Denmark
EPA	Eicosapentaenoic acid
FA	Fatty acid
LHC	Liquid holding capacity
LHC <sub>1</sub>	The amount of liquid left in the mince after centrifugation related to the original liquid content
LHC <sub>2</sub>	The amount of liquid left in the mince after centrifugation related to the dry matter content
LL	Liquid loss
LM	Light microscope
$M_I$	The relative size of water pool I
$M_{II}$	The relative size of water pool II
$M_{III}$	The relative size of water pool III
NMR	Nuclear magnetic resonance
PARAFAC	Parallel factor analysis
PC	Principal component
PCA	Principal component analysis
PLSR	Partial least squares regression
PUFA	Polyunsaturated fatty acids
QI	Quality index
QIM	Quality index method
RMSEP	Root mean square error of prediction
SD	Standard deviation
$T_1$	Longitudinal or spin-lattice relaxation time constant
$T_2$	Transverse or spin-spin relaxation time constant
WHC	Water holding capacity

---

## LIST OF PAPERS

This thesis is based on work reported in the following four publications, referred to in the text by their roman numeral:

- I            **Water distribution in smoked salmon**  
Hanne Løje, Ditte Green-Petersen, Jette Nielsen, Bo M. Jørgensen and  
Kristina N. Jensen  
*Journal of the Science of Food and Agriculture*. (2007) 87(2): 212-217
  
- II           **Quality of cold smoked salmon -  
Reviewing the influence of variations in raw material and technological  
parameters**  
Hanne Løje and Jette Nielsen  
*Journal of Aquatic Food Product Technology*. Submitted.
  
- III          **Changes in liquid holding capacity, water distribution and  
microstructure during chill storage of smoked salmon**  
Hanne Løje, Grethe Hyldig, Kristina N. Jensen, Henrik H. Nielsen and Jette  
Nielsen  
*Journal of the Science of Food and Agriculture*. Submitted.
  
- IV          **Comparison of methods to determine liquid holding capacity in raw and  
smoked salmon and trout**  
Hanne Løje, Grethe Hyldig, Henrik H. Nielsen, Bo M. Jørgensen and Jette  
Nielsen  
*LWT Food Science and Technology*. Submitted.

---

## TABLE OF CONTENTS

PREFACE.....	II
SUMMMARY .....	III
SAMMENDRAG (DANISH SUMMARY) .....	V
ABBREVIATIONS .....	VII
LIST OF PAPERS .....	VIII
TABLE OF CONTENTS .....	IX
INTRODUCTION .....	XI
OBJECTIVES.....	XII
OUTLINE OF THESIS .....	XIII
<b>1. BACKGROUND.....</b>	<b>1</b>
1.1 IMPORTANCE OF COLD SMOKED SALMON FOR DENMARK.....	1
1.2 DEFINITION OF COLD SMOKED SALMON .....	1
1.3 RAW MATERIAL FOR THE PRODUCTION OF COLD SMOKED SALMON.....	1
1.4 THE SMOKING PROCESS .....	2
1.4.1 SALTING.....	2
1.4.2 DRYING AND COLD SMOKING .....	6
1.5 QUALITY PARAMETERS REGARDING RAW AND COLD SMOKED SALMON .....	8
1.5.1 LIQUID HOLDING CAPACITY .....	9
1.5.2 WATER DISTRIBUTION.....	11
1.5.3 LIPID CONTENT .....	11
1.5.4 TEXTURE AND GAPING .....	13
1.5.5 COLOUR.....	15
1.6 SUMMARY .....	16
<b>2. DESIGN AND METHODS.....</b>	<b>18</b>
2.1 DESIGN OF THE STUDY .....	18
2.1.1 DESCRIPTION OF EXPERIMENTS.....	20
2.1.2 SAMPLES, HANDLING AND STORAGE.....	21
2.2 METHODOLOGICAL CONSIDERATIONS .....	26
2.2.1 QUALITY INDEX METHOD (QIM) (EXPERIMENTS 1 AND 2) .....	27
2.2.2 DETERMINATION OF LIQUID HOLDING CAPACITY (EXPERIMENTS 1, 2 AND 3) .....	28
2.2.3 DETERMINATION OF LIPID CONTENT AND FATTY ACID COMPOSITION (EXPERIMENTS 1, 2, 3) .....	31
2.2.4 DETERMINATION OF COLLAGEN CONTENT (EXPERIMENTS 2 AND 3) .....	32
2.2.5 DETERMINATION OF SALT CONTENT (EXPERIMENTS 1, 2 AND 3).....	32



---

2.2.6 DETERMINATION OF PH (EXPERIMENT 2).....	32
2.2.7 DETERMINATION OF WATER DISTRIBUTION BY LOW-FIELD NMR (EXPERIMENTS 2 AND 3) ..	32
2.2.8 MICROSCOPY (EXPERIMENT 2) .....	36
2.2.8.1 Light microscopy.....	36
2.2.8.2 Confocal laser scanning microscopy .....	36
2.2.8.3 Preparation technique .....	37
2.2.8.4 Staining .....	38
2.2.8.5 Microscopes .....	38
2.2.8.6 Samples.....	38
2.2.9 DATA ANALYSIS (TWO-WAY AND THREE-WAY).....	39
2.2.9.1 Principal Component Analysis (PCA) .....	39
2.2.9.2 Partial Least Squares Regression (PLSR) .....	40
2.2.9.3 Validation.....	41
2.2.9.4 Three-way data analysis.....	41
2.2.9.5 PARAFAC .....	43
<b>3. RESULTS AND DISCUSSION.....</b>	<b>45</b>
3.1    QUALITY INDEX METHOD (QIM).....	45
3.2    LIQUID HOLDING CAPACITY .....	46
3.3    RELATIONSHIP BETWEEN METHODS TO DETERMINE LIQUID HOLDING CAPACITY.....	54
3.4    SALT CONTENT.....	59
3.5    VARIATION IN PH.....	60
3.6    VARIATION IN LIPID CONTENT .....	61
3.7    VARIATION IN FATTY ACID COMPOSITION .....	63
3.8    VARIATION IN TOTAL COLLAGEN CONTENT.....	65
3.9    WATER DISTRIBUTION.....	67
3.10   CHANGES IN MICROSTRUCTURE .....	73
<b>4. CONCLUSION AND PERSPECTIVES .....</b>	<b>79</b>
<b>5. REFERENCES .....</b>	<b>81</b>

---

## INTRODUCTION

Cold smoked salmon is a popular product among consumers as well as a commodity of economic importance on the world market. However, during recent years, problems associated with increased liquid loss, discolouration, soft texture, and gaping in cold smoked salmon products have been observed (Espe *et al.* 2002; Rørå and Einen, 2003). Such defects in quality are the reason behind a downgrading of the product, resulting in financial loss for the producers.

The quality of cold smoked products can be influenced by several parameters relating to raw material composition (Espe *et al.* 2004; Rørå *et al.* 1998), processing conditions (Birkeland *et al.* 2003; Birkeland *et al.* 2004a; Birkeland and Bjerkeng, 2005) and “post-processing” parameters (shelf life and storage temperature of the product) (Rørå *et al.* 2003). Additional parameters such as slaughtering methods (Erikson *et al.* 1997; Olsen *et al.* 2006), genetics and rearing conditions (density, feed, temperature, stress etc.) can also have an influence on the product (Rasmussen, 2001). Today the smoking process is still based on traditional principles and methods. The biggest change has been the replacement of wild salmon by farmed salmon as the raw material in the cold smoked production. Farmed salmon differ from wild salmon by e.g. having a higher lipid content due to the rearing strategies.

The liquid holding capacity is an important quality attribute of cold smoked salmon as a decreased liquid holding capacity results in a poorer quality of the product. It is thus of major interest to know how changes in liquid holding capacity occur in relation to raw material, processing and storage of cold smoked salmon.

A suitable measurement of the liquid holding capacity is an important part of the production control. There is no standard method for measuring the liquid holding capacity. Different methods have been used to evaluate the liquid holding capacity in salmonid fish, making it difficult to compare the results. Therefore it is relevant to investigate the suitability of these methods.

---

## OBJECTIVES

This Ph. D. project was initiated as a result of enquires received from the Danish salmon industry regarding quality deficiency (soft texture, discolouration and liquid loss) in industrially produced cold smoked salmon. In particular, liquid loss in the sliced products causes significant financial loss for the producers due to complaints from the retailers.

The main objective of this thesis was thus to study the liquid holding capacity/liquid loss of raw and smoked salmonids as affected by raw material and storage time of the smoked product using a traditional process. In particular this project aimed to answer the following question:

- How are the liquid loss related to the lipid content, raw material variation and storage time?

An investigation into these factors should improve the knowledge about the importance of raw material composition and storage time on the cold smoked product, which in turn should lead to cold smoked products with fewer quality defects. To control the raw material as a part of production control it is advantageous to have a suitable method to determine liquid holding capacity. In research different studies of liquid holding capacity have used different methods and different experimental conditions, and this makes it difficult to compare the results. Thus, the following question was raised:

- Is it possible to suggest methods to determine the liquid holding capacity in salmonid fish to be used in research and production?

---

## **OUTLINE OF THESIS**

This thesis is divided into four chapters. Chapter one gives an introduction to cold smoked salmon, the raw material used for the production of smoked salmon and the cold smoking process. Quality parameters of salmon and cold smoked products are also presented. In chapter two, the design of the study is presented and the methods used are presented and discussed. In chapter three, the results found in this study, i.e. the results from the attached papers, are presented together with unpublished results. Chapter four presents the conclusions and the perspectives.

---

## Chapter 1

### 1. Background

This chapter gives an introduction to the processing of cold smoked salmon with focus on the raw material and the process. In addition, the quality parameters of raw and smoked are described. At the end of the chapter, there is a short summary of the parameters that influence the cold smoked product and finally, the focus of this thesis is given.

#### 1.1 Importance of cold smoked salmon for Denmark

The production of cold smoked salmon is important for Denmark as well as many other north European countries. In Denmark, cold smoked salmon is the most popular smoked fish product, followed by smoked rainbow trout (DFU, 2005). In 2005, the export of cold smoked salmon products from Denmark was 7.947 tonnes, which represents a value of 614 million DKK (the Danish Directorate of Fisheries, 2005) making cold smoked salmon a commodity of considerable economic importance for Denmark.

#### 1.2 Definition of cold smoked salmon

Cold smoked salmon is a traditional product and in Denmark is produced by a large number of smoke houses using traditional or more modern automated production methods. Cold smoked salmon is considered a lightly preserved fish product with a salt content ranging from 3.5 to 6.0 % in the water phase (Hansen *et al.* 1996), a water content between 63 and 70 % (Espe *et al.* 2004; Cardinal *et al.* 2004) and a pH between 5.8 and 6.3 (Hansen *et al.* 1995).

#### 1.3 Raw material for the production of cold smoked salmon

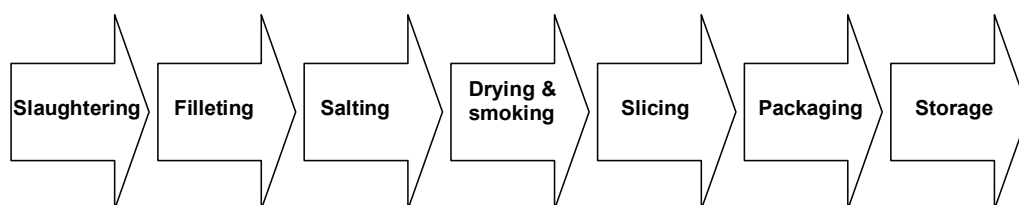
The raw material for the production of cold smoked salmon in Denmark is mainly farmed Atlantic salmon (*Salmo salar*). The farmed salmon are imported from Norway, the Faroe Islands, Scotland, and Chile. There is also a small production of cold smoked salmonid products from imported Pacific *Oncorhynchus* species, from Danish farmed trout (rainbow trout, *Oncorhynchus mykiss*) and from wild Baltic salmon (*Salmo salar*).

---

## 1.4 The smoking process

The cold smoking process has developed from a simple process to a modern industrial process. However the fundamental principles in the smoking technique are still used by the commercial smoke houses. Salting, drying and smoking are used in combination to preserve the fish. Due to the development of refrigeration and packaging technologies it has been possible to reduce the salt content and the smoking time. Thus, nowadays cold smoked salmon appears as a lightly preserved product with less salt, more moisture and less smoke flavour than previously (Hansen, 1995; Rørå, 2003; Birkeland, 2004).

The processing steps vary according to the different recipes used at the smoke houses. In Figure 1.1, a flow chart for the traditional cold smoking process for salmon is shown from slaughtering to final product.



**Figure 1.1.** Flow chart showing the process for the production of cold smoked salmon.

In addition to the steps mentioned in Figure 1.1, freezing can be included in different steps. Smoke houses buy both fresh and frozen raw fish. The smoke houses may choose to freeze their fish either before or after processing due to logistics, market prices, etc. Very often the smoked fillet is frozen short-term to make it easier to slice the smoked fillet (Rørå, 2003). The smoked product might also be stored frozen before being transported to the retail store in order to ease logistics. In this study, freezing was only performed on samples intended for chemical analyses.

The main steps in the cold smoking process are salting, drying and cold smoking. These are described in the following sections.

### 1.4.1 Salting

Salting provides the desired firm texture and salty taste of the flesh (Doe *et al.* 1998).

---

Salting also preserves and increases the shelf life of the product as salting results in lower water activity, thereby lowering microbial activity. The recommended critical limit for salt content of chilled vacuum-packed cold smoked salmon is 3.5 % salt in the water phase to prevent growth and toxin production of *Clostridium botulinum* during chill storage. Different salting methods are applied in the industry e.g. dry, brine or injection salting.

Dry salting has traditionally been used as a salting method for the cold smoking process of salmon and is still frequently used in the industry. The salting time varies between the smoke houses, and can vary from hours (Birkeland, 2004) to several days (FAO/WHO, 1983). The temperature during dry salting is recommended to be below 10°C (FAO/WHO, 1983). Dry salting is done by spreading crystalline salt on the flesh and leaving the fillet to equilibrate. The excess salt is subsequently removed by rinsing.

Brine salting is a process by which the fish is soaked in a salt solution for a certain time. After salting, the fillets are rinsed. Brine concentrations that range from 15 to 24 % salt can be used to achieve at least 3.5 % salt in the water phase (Jittinandana *et al.* 2002). The temperature during brining should not exceed 10°C (FAO/WHO, 1983). The salting time for brine salting depends on several parameters like lipid content, size and thickness of the fillet. For Atlantic salmon (size 3-4 kg) a salting time of 6 hours at 12°C has been reported (Sigurgisladóttir *et al.* 2000a; Sigurgisladóttir *et al.* 2000b).

Several factors can affect the uptake and distribution of salt in salmon fillets that have been dry salted or brine salted. These factors are salt concentrations (Horner, 1992; Jittinandana *et al.* 2002), lipid content in the fillet (Shenderyuk and Bykowski, 1990; Sheehan *et al.* 1996; Cardinal *et al.* 2001; Mørkøre *et al.* 2001; Gallart-Jornet *et al.* 2007), fillet size and thickness (Shenderyuk and Bykowski, 1990; Jittinandana *et al.* 2002; Gallart-Jornet *et al.* 2007), rigor state of the fillet (Shenderyuk and Bykowski, 1990; Wang *et al.* 1998; Wang *et al.* 2000; Rørå *et al.* 2004), salting time (Jittinandana *et al.* 2002; Birkeland and Bjerkeng, 2005), temperature during salting (Diaz *et al.* 1993; Birkeland and Bjerkeng, 2005) and salt to fish ratio (Shenderyuk and Bykowski, 1990; Jittinandana *et al.* 2002).

During dry salting, the salt solution will diffuse into the muscle and water will be extracted



---

from the muscle tissue until equilibrium has been reached. Shrinkage of the muscle fibres of Atlantic salmon fillets due to dry salting has been reported (Sigurgisladóttir *et al.* 2000b; Sigurgisladóttir *et al.* 2001), causing a reduction in the fillet weight after dry salting (Cardinal *et al.* 2001; Mørkøre *et al.* 2001). In brine salting, the fillets are soaked in a solution and as a result, water diffusion is reduced. Thereby, lower weight losses and higher yield are obtained for brine salted fillets compared to dry salted fillets (Cardinal *et al.* 2001).

The diffusion of salt is an important issue during salting and the lipid content may be a limiting factor for the diffusion (Wang *et al.* 2000; Gallart-Jornet *et al.* 2007) either by replacing the aqueous phase that serves as a vector for transfer during the salting step or by acting as a physical barrier. Therefore, the yield after salting depends on the lipid content/water content in the fillets as the transfer of the salt solution decreases with increased lipid content (Sheehan *et al.* 1996; Wang *et al.* 2000) and thereby a decreased weight loss is observed during salting and smoking (Rørå *et al.* 1998; Mørkøre *et al.* 2001). The rate of the salt diffusion is temperature dependent and increases as the temperature increased (4-40°C) (Diaz *et al.* 1993; Corzo and Bracho, 2004).

The dry salting step itself and the conditions applied during the process affect several parameters e.g. colour, liquid holding capacity, texture and yield in the final smoked fillets (Birkeland *et al.* 2004a; Birkeland and Bjerkeng 2005). Cardinal *et al.* (2001) found no colour difference between samples which were dry salted or brine salted. Birkeland and Bjerkeng (2005) found that the salting temperature and salting time of brine or dry salting affect the colour of cold smoked Atlantic salmon. The smoked fillets which were brine salted or dry salted at low temperature (4°C) were significantly more light and yellow compared to fillets salted at 10°C. Upon increasing the salting time from 6 to 12 hours the smoked fillets became less light and red. A further increase of the brining time from 12 to 24 hours did not have any significant effects on surface colouration of the smoked fillets. The colour parameters of smoked fillets were more affected by the brine concentration than by the brining time and the temperature. The fillets salted in a brine concentration of 50 % saturated salt solution were significantly less red than fillets salted in a brine concentration of 100 %. The brine concentration did not affect the lightness and

---

yellowness of the smoked fillets (Birkeland and Bjerkeng, 2005). Birkeland *et al.* (2004a) found that fillet processed according to dry salting were firmer and more elastic than fillets which were processed according to injection-salting. Birkeland and Bjerkeng (2005) found that increasing salting temperature (4 to 12°C) significantly increased the weight loss by 1.5 % units and increased salting time (6 hours vs. 24 hours) significantly increased the weight loss by 3.2 % units. Other studies (Cardinal *et al.* 2004; Mørkøre *et al.* 2001) have found similar weight losses following dry salting.

The injection salting method was originally developed by the meat and poultry industry. The injection salting method is now frequently used by producers of cold smoked products. During injection salting, brine is injected through needles directly into the muscle tissue by means of pressure (Birkeland *et al.* 2003). The applied pressure serves to distribute the brine among the muscle fibres. Hence, the salt uptake during injection salting is not solely dependent on diffusion. Deposits of brine will be formed in close proximity to the injection sites and salt will be distributed throughout the fillets by diffusion during the following production unit operations (drying and cold smoking) and vacuum-storage (Birkeland *et al.* 2003; Birkeland, 2004). The salt content in smoked fillets is affected by the brine injection pressure, needle speed, needle density and injection direction. In addition, the concentration of the brine injected will affect the salt content in the smoked fillet as the amount of salt injected into the muscle tissue is almost proportional to the amount of brine injected (Birkeland, 2004). A high pressure (above 0.4 MPA) may cause damage to the muscle structure, e.g. gaping (Birkeland *et al.* 2003).

By adapting the injection salting method, production time is saved and a higher processing yield is obtained (Birkeland *et al.* 2003; Birkeland *et al.* 2004a; Birkeland *et al.* 2004b) compared to the dry salting and brine salting methods. The injection salting method leads to products that differ in quality traits compared to dry salted smoked products (Birkeland *et al.* 2004a; Birkeland *et al.* 2004b). A higher gaping score and softer texture have been reported for salt injected salmon fillets compared to dry salted salmon fillets (Birkeland *et al.* 2004a). Birkeland *et al.* (2004b) found that the dry salting technique affected the variation in colour characteristic more than the injection salting technique, which indicated that the injection salting technique gives a more homogenous smoked product with respect

---

to colour characteristics than that obtained after dry salting.

#### **1.4.2 Drying and cold smoking**

The conditions used for drying the salmon fillets vary substantially from producer to producer, but usually the temperature is in the range of 15-26°C and the relative humidity is in the range of 55-70 % (Birkeland *et al.* 2003; Birkeland *et al.* 2004a; Birkeland *et al.* 2004b). Drying removes water from the fillet and thus decreases the water content and the water activity of the product.

Cold smoking is usually performed within a temperature range of 20 to 30°C for 2 to 12 hours at a humidity of 60-75 % (Hansen 1995; Birkeland *et al.* 2003; Birkeland *et al.* 2004a; Birkeland *et al.* 2004b). The temperature in the oven must never exceed 30°C, as there should be no “cooking” of the fish (Doe *et al.* 1998). Cold smoking can be performed by use of wood-chips for smoke generation. A broad range of chips from hardwoods like oak, hickory, cherry and beech are usually preferred (Birkeland, 2004). During smoking, the product absorbs volatile compounds from the smoke and loose water. The characteristic flavour and taste of smoked fish is mainly due to the phenols present in the smoke (Maga, 1987; Doe *et al.* 1998).

The yield after smoking is an important factor for economical success in the smoking industry. Weight loss during smoking is mainly due to the evaporation of surface water and the diffusion of water within the flesh towards the surface of the fillet. The diffusion is affected by the quantity of water available in the flesh, and leaner fillets with a high water content will lose more water than fatty fillets with a lower water content (Rørå *et al.* 1998; Cardinal *et al.* 2001; Mørkøre *et al.* 2001). Injection salting gives a higher yield after smoking (in the range of 92-102 %) (Rørå *et al.* 1998; Birkeland *et al.* 2003; Birkeland *et al.* 2004a; Birkeland *et al.* 2004b) than dry salting, which gives yields in the range of 82-91 % (Sigurgisladóttir *et al.* 2000a; Sigurgisladóttir *et al.* 2000b; Cardinal *et al.* 2001; Regost *et al.* 2004; Rørå *et al.* 2004; Birkeland and Bjerkeng 2005). The lower smoking yield obtained for dry salted fillets compared to injection salted fillets is due to loss of water during the dry salting and the supply of brine to the fillets during injection salting (Birkeland, 2004).

---

The duration of the salting step has shown to affect the smoked fillet yield as an increase in the salting time (6 to 24 hours) decreases the smoked fillet yield significantly (Birkeland and Bjerkeng, 2005). The smoking temperature affects the yield of smoked Atlantic salmon fillets (Sigurgisladóttir *et al.* 2000a; Sigurgisladóttir *et al.* 2000b; Cardinal *et al.* 2001; Birkeland and Bjerkeng, 2005), and smoking at a temperature of 30°C caused the fillet yields to be higher than smoking at a temperature of 20°C. However, Birkeland *et al.* (2003; Birkeland *et al.* 2004a) found that smoking at a high temperature (30°C) compared to a low temperature (20°C) led to a lower smoking yield. Increasing yield of smoked Atlantic salmon fillets with increasing fillet lipid content has been reported (Cardinal *et al.* 2001; Torrissen *et al.* 2001). The differences in yield after smoking between fillets with high lipid contents may be explained by reduced dehydration during the processing of fillets with a high lipid content (Sigurgisladóttir *et al.* 2000a; Torrissen *et al.* 2001).

Temperature during processing has a significant effect on the quality characteristics of smoked salmon. By increasing the temperature during smoking, the colour parameters are altered (Birkeland *et al.* 2004b; Cardinal *et al.* 2001). A reduction in redness was found to be higher for fillets smoked at 20°C than at 30°C, whereas changes in lightness, yellowness and hue were not affected by smoking temperature (Birkeland *et al.* 2004b). A high smoking temperature (30°C) gave higher firmness and elasticity in smoked fillets compared to smoking at 21°C (Rørå *et al.* 2005b), which may be due to a higher drying rate at a higher temperature and thereby a higher water loss, which subsequently gives a firmer and more elastic texture (Jittanandana *et al.* 2002). Rørå *et al.* (2005b) investigated the effect of cold smoking temperature (20 °C and 30 °C) on liquid holding capacity in smoked fillets and found no significant effect of smoking temperature on the amount of exudates, but a positive regression was found between liquid loss and smoking temperature in smoked fillets from salmon fed dietary Peruvian fish oil as sole oil.

To conclude, the recommended salt content of chilled vacuum-packed cold smoked salmon is 3.5 % salt in the water phase. Different salting methods are applied in the industry e.g. dry, brine or injection salting. Several factors can affect the uptake and distribution of salt in salmon fillets. The highest yield is obtained after injection salting, while injection

---

salting gives a softer texture and gaping in the smoked fillets compared with dry salting and brine salting. Dry salting affects the colour parameter more than the injection salting. The applied conditions during smoking affect the colour parameter and texture. High smoking temperature gives a firmer and more elastic texture and a reduced redness in smoked salmon compared with smoking at low temperature. Long smoking time decreases the yield after smoking.

### **1.5 Quality parameters regarding raw and cold smoked salmon**

Quality is one of the most frequently used words relating to food. However, it is exceptionally difficult and complicated to define quality in terms that are widely understood (Meiselman, 2001). Quality can be regarded as a concept and Bremner (2000) has described a new approach for defining the quality of food materials in usable common terms. This is achieved by a nominal hierarchy, where the highest level is an overall concept of quality. Below that is the very general definition e.g. that of the International Standards Organisation (ISO) that covers all aspects of quality, and below that a specific definition constructed to suit the material in question under given circumstances. The specific definitions are linked to criteria, methods, values, and specifications. In practice, the concept and the general definition would be taken for granted and only the specific definitions, criteria and methods are used. In this work, the quality parameters of raw and smoked salmon will mainly be related to properties and attributes measured by standard and instrumental methods.

Sigurgisladóttir *et al.* (1997) have reviewed salmon quality, reporting on the main quality parameters of raw salmon based on literature studies and interviews with companies throughout Europe. The quality parameters of particular importance were lipid content, composition and distribution of lipids in the fillet, colour intensity, distribution of the colour in the fillets and texture (firmness and gaping). Other important cited parameters were white stripes (myocommata and connective tissue), bleeding, blood stains, marbling and melanin (Sigurgisladóttir *et al.* 1997). Koteng (1992) found in a survey that problems with texture, blood stains and colour of the skin were the reason why Norwegian salmon was not of the expected quality. Another important quality issue is the liquid holding capacity as a decreased liquid holding capacity can lead to liquid leakages from the

---

products, which is an important quality problem in products available on the commercial market (Cardinal *et al.* 2004). The most important quality parameters will be discussed in the following sections.

### **1.5.1 Liquid holding capacity**

The liquid holding capacity of fish muscle is of major importance with respect to both commercial value and consumer acceptance (Elvevoll *et al.* 1996). A reduced liquid holding capacity makes the fillets more prone to liquid leakage during smoking or afterwards during slicing and vacuum storage. Accumulated liquid leakages in vacuum-packaged smoked salmon have a negative effect on the product appearance (Birkeland, 2004).

The liquid holding capacity is defined as the ability of a given structure to prevent water and/or lipids from being released from the structure, and it refers therefore to both water and lipid holding capacity (Hermansson, 1986). For lean fish, the term water-holding capacity is generally used.

The lipid holding property is a complicated parameter as it relates to both lipid that is emulsified or entrapped in a protein matrix and to the solid lipid making an important contribution to the muscle structure (Hermansson, 1986).

The liquid holding capacity of fish muscle can be influenced by factors such as salt concentration (Ofstad *et al.* 1996), pH (Regenstein *et al.* 1984; Ofstad *et al.* 1995; Ofstad *et al.* 1996), storage conditions (Mackie, 1993; Nilsson and Ekstrand, 1994; Rørå and Regost, 2003) and heat (Ofstad *et al.* 1993; Ofstad *et al.* 1995; Ofstad *et al.* 1996; Rørå and Regost, 2003), all of which influence the structure and conformation of myofibrillar and connective tissue proteins.

Most of the water in the muscle is present in the myofibrils. The myofibrillar proteins are primarily responsible for the binding of water in the muscle. Uptake of water occurs by the entry of water into the myofibrils as they swell laterally by expansion of the filament lattice. During salting, salt causes swelling of the muscle and an increase in the water

---

holding capacity. The maximum swelling is reached for salt concentrations at 1M (~ 6 %) (Offer and Trinick, 1983). Higher concentrations will reduce the swelling effect and decrease the liquid holding capacity of the fish muscle (Hamm, 1986; Shomer *et al.* 1987; Jittinandana *et al.* 2002).

The pH is an important factor for the liquid holding capacity. The minimum liquid holding capacity is at the isoelectric point, where the myosin or actomyosin has zero net charge. At lower or higher pH values, the proteins have a positive or a negative net charge and the liquid holding ability increases (Morrissey *et al.* 1987). Ofstad *et al.* (1995) found that an increase in pH from 6.0 to 7.0 (in a model system) greatly influenced the liquid holding capacity as the liquid loss was reduced. The effect of pH is influenced by the salt concentration and the temperature (Ofstad *et al.* 1995).

Storage and processing of fish can influence the liquid holding capacity of the muscle. Such procedures include transportation, storage, freezing and thawing, drying, mincing, salting, smoking, canning and cooking (Hamm, 1986).

Heat-induced structural changes in muscle are related to decreased liquid holding capacity. Ofstad *et al.* (1993) found that the liquid loss was almost constant between 5 and 20°C. At higher temperature the liquid loss (mainly water) increased rapidly as a function of temperature and heat-induced shrinkage of the myofibrillar proteins and increased water loss were seen (Ofstad *et al.* 1993; Ofstad *et al.* 1995). A temperature between 20 and 30°C is used for cold smoking salmon. At temperatures between 20-35°C, the main structural changes appeared in the endomysium surrounding the muscle cells. The endomysial layer swelled and melted collagen fibres filled the widened collagen spaces. Therefore, a liquid loss between 20 and 35°C may be due to the denaturation of collagen altering the physical properties of the pericellular layer, which represents a physical barrier to release the fluid (Ofstad, 1995).

In addition, biological factors such as chemical composition, biological state e.g. spawning and starvation (Love, 1988), and post-mortem processes, e.g. rigor, can influence the fish flesh quality and thereby influence the liquid holding capacity.



---

To conclude, the liquid holding capacity of smoked salmon is affected by several parameters: pH, salt, heating as well as storage time and storage temperature after smoking.

### **1.5.2 Water distribution**

Water is the main constituent of muscle and many important quality parameters such as liquid holding capacity, storage stability and texture are related to the interaction of water with protein and the distribution of water in the muscle tissue (Fjelkner-Modig and Tornberg, 1986; Offer *et al.* 1989; Hills, 1998; Ruan and Chen, 1998). Together, water and lipid make up about 80 % of the fish muscle and they are inversely related. Thus, variation in lipid content leads to changes in the water fraction and that may be reflected in the water distribution. This was shown for herring, where water distribution varied according to the lipid content (Jensen *et al.* 2005).

Many of the functional properties are related to the interactions between water and protein (Schnepf, 1989). The muscle water can be regarded as distributed into several populations or “pools” depending on the mobility of the water molecules e.g. how tight the water is bound or entrapped to structural elements of the cells (e.g. myofibrillar protein) (Ruan and Chen, 1998). A fraction of the water is structurally bound to the protein (structural water), whereas the main part of the water has different degrees of association to the proteins (Morrissey *et al.* 1987; Schnepf, 1989). The distribution of water between these pools depends on changes in the chemical composition and the physical structure (Hills, 1998; Ruan and Chen, 1998) due to e.g. processing and/or storage.

To conclude, water and lipid are inversely related and the sum of these two constituents accounts for about 80 % of the fillet. Knowledge about water distribution in raw and cold smoked salmon muscle may provide important information about the relationship between changes in the raw material properties and the quality differences in the final product.

### **1.5.3 Lipid content**

The lipid content, its composition and distribution in the fillets are important quality

---

parameters in salmon (Sigurgisladóttir *et al.* 1997). Increased lipid content decreases the smoked fillet yield (Rørå *et al.* 1998; Mørkøre *et al.* 2001; Birkeland *et al.* 2004a). The effect of high lipid content on the sensory parameters shows contradictory results. Rørå *et al.* (1998) found no effect, whereas Sheehan *et al.* (1996) found a significant effect of lipid content on the sensory attributes (oiliness, texture, colour and gaping) after 24 days after smoking. Robb *et al.* (2002) found that an increased lipid content led to a decreased firmness and an increased oily taste in smoked salmon. A high lipid content affects the colour of dry salted smoked fillets (Sheehan *et al.* 1996; Einen *et al.* 1999; Birkeland *et al.* 2003) as fillets with a high lipid content are more light and yellow than fillets with a low lipid content (Birkeland *et al.* 2003). In the study by Birkeland *et al.* (2003), the redness was not affected by the lipid content. However the opposite was seen by Sheehan *et al.* (1996) and Einen *et al.* (1999).

A high lipid content has been reported to lead to a decreased liquid holding capacity. Mørkøre *et al.* (2001) found a negative correlation between lipid content (between 14.5 and 21.8 %) and liquid holding capacity (determined by the centrifugation method by Gomez-Guillén *et al.* 2000) in smoked salmon fillets. Birkeland *et al.* (2004a) found a reduced liquid holding capacity (measured by a higher lipid loss) for smoked salmon with high lipid contents ( $20.2 \pm 1.1$  %) as opposed to smoked salmon with low lipid contents ( $16 \pm 1.0$  %).

Farmed Atlantic salmon of market size has a lipid content between 6 and 22 %, with 15-16 % as an average measured in the Norwegian quality cut (Sigurgisladóttir *et al.* 1997; Rørå *et al.* 1998). The total lipid content can vary a lot and variation in lipid contents between individuals is high (Bell *et al.* 1998; Refsgaard *et al.* 1998). Even in the same batch there can be considerable individual variation in lipid content (Rørå *et al.* 1998). In addition, the lipids are not equally distributed in the fillet (Hardy and King 1989; Aursand *et al.* 1994; Refsgaard *et al.* 1998; Katikou *et al.* 2001). The highest lipid content is found in the dorsal lipid depot, the belly flap area and the red muscle, all of which contain three times as much lipid as the white muscle (Aursand *et al.* 1994).

The lipid content in farmed Atlantic salmon is influenced by several parameters such as the

---

lipid content and the lipid composition of the feed (Lie *et al.* 1993; Bell *et al.* 1998; Hemre and Sandnes, 1999), annual variations (Aknes *et al.* 1986; Mørkøre and Rørvik, 2001) and the body weight of the fish (Storebakken *et al.* 1991; Shearer *et al.* 1994; Torrissen *et al.* 2001). Other parameters, e.g. genetic strain, environment and sexual maturation, may also affect the lipid content (Gjedrem, 1997).

To conclude, there is high variation in lipid content of salmon fillets between individual fish within a population and the lipid content can range from 6 to 22 % in farmed salmon. The lipid content in fillet affects several quality parameters in the smoked salmon e.g. yield, texture, colour, sensory attributes and liquid holding capacity. Thus, knowledge of the lipid content is of importance to assure a consistent quality.

#### **1.5.4 Texture and gaping**

The texture of fish is an important quality characteristic and fillets with soft texture and/or gaping can cause considerable economical losses due to quality downgrading (Lavéty *et al.* 1988; Michie, 2001). The term gaping is used to describe the gaps, tears or slits that are found in post mortem fish flesh (Bremner, 1999), and muscle gaping occurs when the connective tissue fails to hold the blocks of muscle together (Lavéty *et al.* 1988; Love, 1988). Gaping in salmon makes the flesh unsuitable for the production of sliced products. Products with gaping are less presentable, and since the flesh is soft, the texture is less acceptable (Bremner, 1999).

The texture of fish muscle is influenced by season (Mørkøre and Rørvik, 2001), the collagen content (Sato *et al.* 1986; Bremner, 1992), post mortem factors, pH (Einen *et al.* 1999) the lipid content and the distribution of muscle lipid (Regost *et al.* 2004). During the cold smoking process, the texture of the fish fillet will change. The processing parameters that affect the textural characteristics are the processing temperature, salt/ionic strength and pH, since these have an influence on the structure and conformation of the myofibrillar and connective tissue proteins (Dunajski, 1979). Cold smoked salmon products are firmer and more elastic compared to raw, unprocessed fillets (Sigurgisladóttir *et al.* 1999; Sigurgisladóttir *et al.* 2000a).

---

Salmon processors have blamed a high lipid content in farmed Atlantic salmon for causing increased muscle gaping in the smoked fillets (Sheehan *et al.* 1996), which can result in difficulties when slicing the smoked fish (Eckhoff *et al.* 1998). However, Birkeland *et al.* (2003) found no evidence of lipid content in fillet affected the gaping scores in smoked Atlantic salmon following dry- or injection salting, whereas Sheehan *et al.* (1996) found that gaping was most severe in smoked fillets which had been fed on a high lipid diet. Gaping is influenced by the salting method as more muscle gaping was found in injection-salted than dry salted fillets (Birkeland *et al.* 2003). The texture properties of smoked fillets are affected by fillet lipid content as well as by the salting method. Dry salted fillets were firmer and more elastic than fillets subjected to injection salting. The observed difference in textural characteristics was due to different water contents in the smoked fillets (Birkeland *et al.* 2004a). Sheehan *et al.* (1996) found that smoked fillets fed with a low lipid diet (21 %) were significantly softer than fillets of fish fed with a medium (25 %) or a high lipid diet (30 %).

Collagen is the major component of connective tissue. The connective tissue forms a supporting network through the whole fish muscle. Therefore, the connective tissue has a significant influence on the functional and rheological properties of the muscle and is the main contributor to the tensile strength in the muscles (Sikorski *et al.* 1990). Gaping and texture of fish muscles are influenced by the total collagen content. High collagen content is associated with firmer texture and less gaping incidence (Sato *et al.* 1986; Hatae *et al.* 1986). Raw fish flesh from most fish species softens after a few days of chilled storage (Sato *et al.* 1991). Histological studies have shown that the rapid softening of fish flesh is caused by weakening of the connective tissue resulting from a disintegration of thin collagen fibrils (Hallett and Bremner, 1988; Ando *et al.* 1991; Ando *et al.* 1992). Fish collagen contain less proline and hydroxyproline than mammalian collagen so fish collagen is thermally less stable and more easily soluble than mammalian collagen. The denaturation and shrinkage temperatures of fish collagen are near 20°C and 40°C, respectively (Sikorski *et al.* 1984).

The muscle fibres in fish fillets run approximately from the front of the fillet to the tail in adjacent muscle blocks called myotomes. The myotomes are one cell (muscle fibre) deep

---

and are separated from the muscle fibres of the adjacent myotomes by collagen sheets termed the myocommata (Bremner and Hallett, 1985). Within each myotome, the muscle fibres run approximately parallel to each other and are attached to the myocommata of the fibre ends (Bremner and Hallett, 1985; Love 1970; Bremner, 1992). The individual muscle fibre is comprised of bundles of myofibrils. The cell wall of the individual muscle fibre is referred to as the sarcolemma. The myofibrils consists of thick filaments, myosin and thin filaments, which are composed of actin, tropomyosin and troponin (Howgate, 1979).

The individual muscle fibre constitutes the muscle cell, which is surrounded by a cell membrane called the sarcolemma. Outside or surrounding this is the basement membrane and the endomysial layer of fine collagen fibres. The endomysium runs into the surrounding perimysium which in fish emerges from the myocommata (Bremner and Hallett, 1985). Although the endomysium, perimysium and myocommata are considered to be discrete areas of the extracellular matrix, they join to form a single weave.

To conclude, the texture of fish muscle changes during cold smoking process and becomes firmer and more elastic. The texture is influence by the lipid content and collagen content as well as post mortem factors and processing. Contradictory results have been found regarding the effect of lipid content on gaping.

#### **1.5.5 Colour**

The colour of a product is the first impression a consumer has of a given product, and if the consumer dislikes the colour, then the other quality parameters such as texture or flavour are not likely to be judged at all (Francis, 1995). Thus, the colour of the flesh is among the most important quality characteristics of salmon (Sigurgisladóttir *et al.* 1997). The red colour of salmon flesh is due to carotenoids, and farmed salmon derive the colour from the carotenoid astaxanthin, which is added to the feed (Skrede and Storebakken, 1986a; Bjerkeng, 2004). Another carotenoid, canthaxanthin, can also be used either alone or in combination with astaxanthin. However, restrictions on the use of canthaxanthin have been introduced by the EU (Bjerkeng, 2004).

Problems related to discolouration are occasionally observed in salmon and salmonid fish

---

species. Several kinds of colour deficiencies exist and include poor pigmentation, discolouration, partly or complete depigmentation and zebra stripes (Torrissen, 1995). Several factors can influence the colour, e.g. the concentration and type of the pigment in the feed (Skrede and Storebakken, 1986b; Bjerkeng, 2000), dietary lipid level (Regost *et al.* 2001), the size of the fish, the stage of sexual maturity (Aknes *et al.* 1986; Bjerkeng *et al.* 1992), the lipid content in the fish (Rørå *et al.* 1998) and processing (Birkeland and Bjerkeng, 2005). A general trend is that cold smoked fillets are less red and less light, but more yellowish compared to the raw material (Rørå *et al.* 1998; Cardinal *et al.* 2001; Birkeland *et al.* 2004a).

To conclude, the colour of smoked salmon is influenced by several factors such as processing parameters and contents of carotenoid and lipid. It is important to be aware of how these parameters affect the colour as colour is an important parameter for cold smoked salmon.

## **1.6 Summary**

Cold smoked salmon is a very popular product among consumers and is also of considerable economic importance. However, during recent years the salmon smoking industry has faced problems such as liquid loss, soft texture and discolouration of the smoked product.

The smoking process is still based on traditional principles and methods. The main steps in the cold smoking process are salting, drying and cold smoking, and numerous procedures are utilized by different smoke houses. Processing conditions are important factors that affect the quality of the final product and the processing yield.

The quality of the cold smoked product can be influenced by many other factors. The lipid content in the raw material can vary a lot and in addition, high variation between individual fish has been reported. The lipid content is an important parameter, which can affect yield, sensory properties, colour, texture and liquid loss. Lipid and water contents are inversely related and changes in lipid content will affect the water content. Water and its interactions with protein affect several functional parameters such as liquid holding capacity and

---

texture.

The liquid holding capacity is an important parameter as low liquid holding capacity can lead to higher liquid loss. The liquid holding capacity can be influenced by several parameters, e.g. salt, pH, lipid content, chemical composition and post-mortem process. Soft texture and/or gaping are of major importance as they can lead to difficulties in slicing the products. The texture depends on several parameters e.g. collagen content, season, post mortem factors, pH and lipid content. Several kinds of discolouration have been reported and several parameters may affect the colour, e.g. processing conditions, concentration of pigment and lipid content.

Within recent years several studies have been carried out into the factors that influence the cold smoking product. However, problems are still observed with respect to liquid loss, soft texture and discolouration in cold smoked products. The production of farmed salmon has grown substantially during the recent years and the lipid level in farmed fish has increased. Thus, the lipid content and its distribution in the fish may be important parameters regarding the problems with quality of cold smoked salmon.

The main objectives of this thesis were to study the liquid holding capacity/liquid loss of raw and smoked salmonids as affected by raw material and chill storage of the smoked product.



---

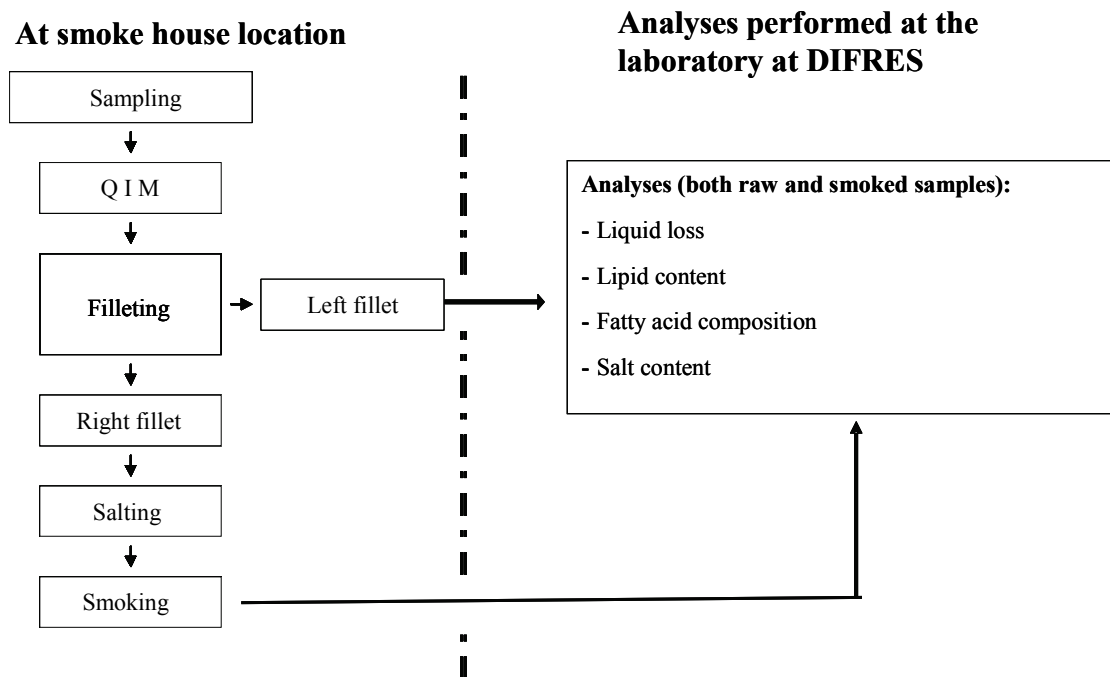
## Chapter 2

### 2. Design and methods

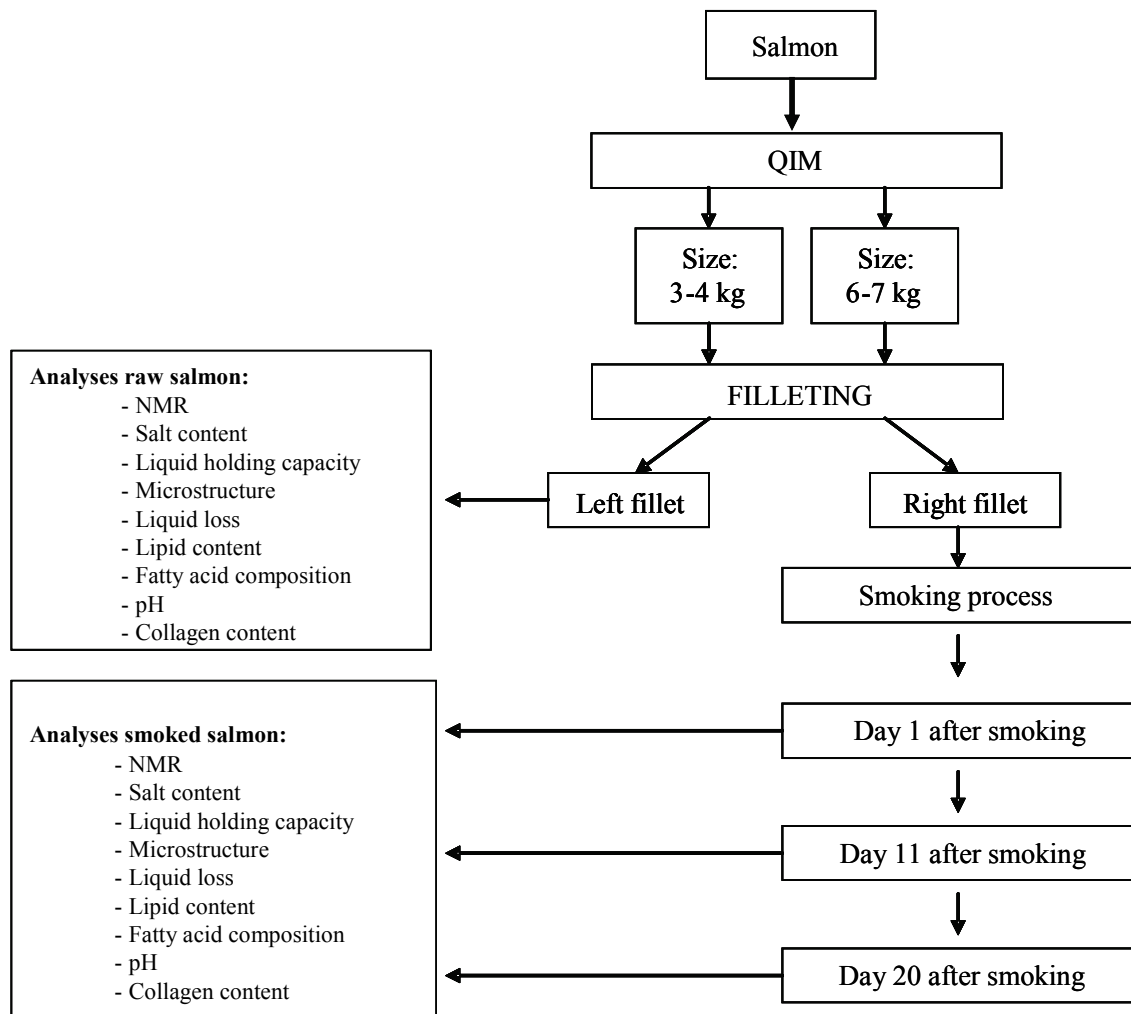
This chapter gives an introduction to the design of the study, the experiments and the methods used in this work. In section 2.1, the design of the study and an overview of the experiments are given, including a description of sampling, handling and storage. In section 2.2, a brief description of the used methods is given.

#### 2.1 Design of the study

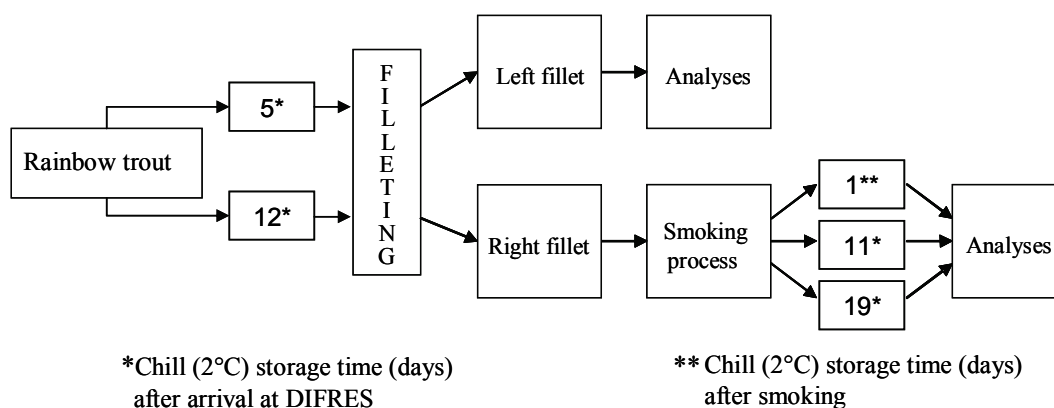
The objective was to investigate the effect of raw material and chill storage on quality parameters of cold smoked salmon. In order to investigate this, three experiments were carried out. In Figure 2.1 to 2.3 overviews of the experimental designs are given.



**Figure 2.1.** Overview of sampling and analyses in experiment 1. The dotted line indicates that the QIM, filleting, salting and smoking took place at the commercial smoke house, while the cutting of the fillet into smaller pieces and analyses were carried out at the laboratory at DIFRES.



**Figure 2.2.** Overview of sampling and analyses in experiment 2.



**Figure 2.3.** Overview design for experiment 3.

### 2.1.1 Description of experiments

The three experiments will be described briefly in the following. All three experiments were carried out in collaboration with the Danish cold smoking industry. In experiment 1, the salting and smoking processes were done at a commercial smoke house according to their recipe, whereas the sampling and analyses were performed at the laboratory at the Danish Institute for Fisheries Research (DIFRES). In experiments 2 and 3, the salting and smoking processes as well as the analyses were done at the laboratory at DIFRES.

#### Experiment 1

The purpose of experiment 1 was to characterize the variation in the raw material and the smoked product in a commercial smoke house. The smoke house was instructed to follow its standard procedure. The aim was to determine the variation in the normal production in order to reveal any critical points according to raw material and/or processing, which could have an influence on the final product. For a period of four weeks, samples were taken out twice a week from the production and analysed for chemical composition and functional properties.

#### Experiment 2

The purpose of experiment 2 was to study the influence of the size of the fish on the smoked product. The salmon came from two farms in Norway and there were two sizes of

---

fish from each farm. Thus four groups were made. All the fish went through the same cold smoking process. The fish were salted and smoked at the laboratory at DIFRES according to in-house recipes (referred to as in-house smoked). The smoked fillets were chill stored for up to 20 days. Three times during the storage period, samples were taken out for analysis. Three fish from one of the farms (farm 2) were dry salted and smoked in a traditional oven at a commercial smoke house in Denmark. Both the salting and the smoking process were longer than for the in-house smoked samples. Afterwards, the fillets were sent to the laboratory at DIFRES, where they were analysed for chemical composition and functional properties in the same way as the in-house smoked salmon. Thereby, an indication of the influence of different processing could be obtained using the same raw material. The samples are referred to as commercial samples.

### **Experiment 3**

The purpose of experiment 3 was to study the influence of different feeding strategies for rainbow trout on the smoked rainbow trout product. Compared to experiment 2, rainbow trout were used instead of salmon and in addition a chill storage period of the raw fish prior to filleting and processing was included in the design to evaluate the effect of chill storage of raw fish on the smoked rainbow trout product. The rainbow trout were salted and smoked at the laboratory at DIFRES according to in-house recipes in the same way as the salmon in experiment 2. The smoked fillets were chill stored for up to 19 days. Three times during the storage period samples were taken out for analysis.

#### **2.1.2 Samples, handling and storage**

In experiments 1 and 2, Atlantic salmon (*Salmo salar*) farmed in Norway were used as raw material. In experiment 3, rainbow trout (*Oncorhynchus mykiss*) farmed in Denmark was used. The rainbow trout were chosen as raw material because information from egg to fish could be obtained e.g. feeding strategies, maturity etc. In all three experiments, the left fillets were used for analysis of raw material and the right fillets were processed into smoked fillets and analysed as smoked products.

### **Experiment 1**

Since the purpose of experiment 1 was to obtain an overview at what level variations can

---

be expected in a commercial process, the commercial smoke house was told to follow the normal procedure for filleting, salting and smoking. The only requirement was that the size of the fish should be the same and only farmed salmon from Norway was to be used during the whole period.

For a period of four weeks, salmon samples were taken out twice a week. The first sampling day in a week was the day when the fish arrived at the smoke house (analysis day A). The second sampling day in the same week was 2-3 days after the first day (analysis day B). Meanwhile the fish were stored in a chill storage room at 1°C at the smoke house. Samples taken out from the same week were from the same batch. One box of raw salmon was used on the first sampling day (analysis day A) and another box of raw salmon from the same batch was used on the second sampling day (analysis day B). In total, four different batches were analysed and three of them (week 2 to 4) were from the same supplier and the batch from week 1 was from another supplier.

Before filleting, the whole fish were evaluated by the quality index method (QIM). After filleting, the left fillets were used for analysis of raw material. The right fillets were dry salted for one to four days. During dry salting, the fillets were kept in a chill room at the smoke house at a temperature of 2°C. After that, the right fillets were dried for two hours and smoked in an automatic oven for approx. 9 hours at 20 to 22°C with a relative humidity of 60 %.

In the laboratory at DIFRES, both the left and the right fillets were cut into the same pieces for analysis; the piece under the dorsal fin (above the lateral line) was used to determine liquid holding capacity and the piece anterior to the dorsal fin (The Norwegian Quality Cut) was used to determine chemical composition.

### **Experiments 2 and 3**

In experiment 2, four groups of Atlantic salmon (*Salmo salar*) were analyzed. The fish came from two farms in Norway with two sizes (3-4 kg and 6-7 kg) of fish from each farm. The fish arrived at the laboratory at DIFRES six days after being slaughtered.

In experiment 3, rainbow trout (*Oncorhynchus mykiss*) from two different fish seafarms in Denmark were used. The fish arrived at the laboratory at DIFRES day 1 (group 4) and day 2 (group 1-3) after slaughtering. The fish were divided into four groups according to feeding strategies, maturity and farm. The feeding of the fish was either normal or restricted and the states of maturity were either mature, immature or in one case a mix (Table 2.1). Fish from farm 2 were slaughtered one day before the other fish. There were eight fish in each group. Upon arrival each group was divided into two subgroups with four fish in each subgroup. The fish were iced again before storage. Subgroup A was stored at 2°C until 5/6 days after slaughtering, and subgroup B was stored at 2°C until 12/13 days after slaughtering.

**Table 2.1.** Overview samples in experiment 3

	Feeding	Number of fish	Maturity	Farm	Strain	Chill storage before processing Number of fish A <sup>1</sup> or B <sup>2</sup>
Group 1	Restrictive	8	Mature	1	Fausing	A (short) 4 B (long) 4
Group 2	Restrictive	8	Immature	1	Fausing	A (short) 4 B (long) 4
Group 3	Normal	8	Mature/immature	1	Trend	A (short) 4 B (long) 4
Group 4	Normal	8	Mature	2	Trend	A (short) 4 B (long) 4
<b>Total</b>						<b>32</b>

1: A (short): The fish were stored for 5-6 days after slaughtering and before processing

2: B (long): The fish were stored for 12-13 days after slaughtering and before processing

The salmon (experiment 2) were evaluated by the quality index method (QIM) on the day of arrival at the laboratory at DIFRES.

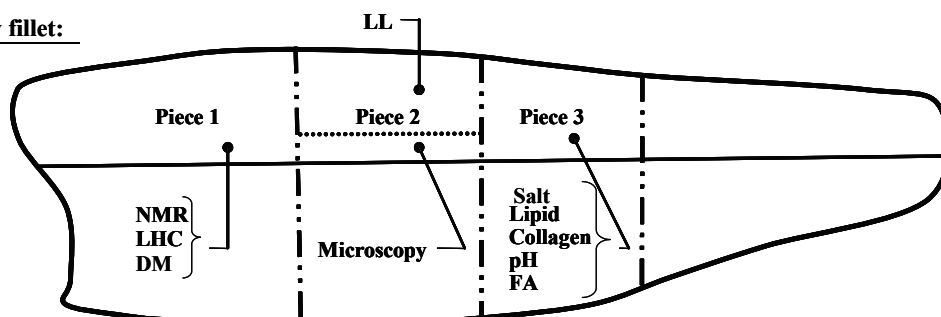
The salmon and the rainbow trout (after chill storage of the raw fish) were processed in the same way. After filleting, the left fillets were kept as raw (unprocessed) and used for analysis of raw material. The left fillets were cut into smaller pieces (Figure 2.4). The right fillets were processed into smoked fillets.

---

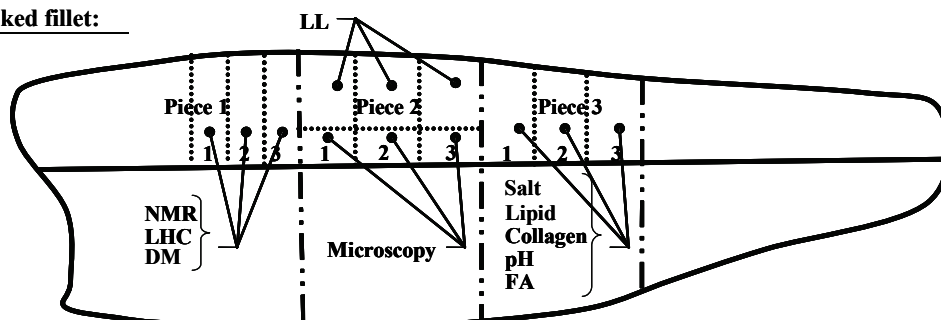
The right fillet was dry salted according to an in-house recipe. The fillets were dry salted (60 g salt per kg fillet) for five hours for small fish (size 3-4 kg) and eight hours for the large fish (size 6-7 kg) at room temperature. Fine salt with a grain size 0.6-0.13 mm (Brøste, Denmark) was used and spread over the fillet side and the fillets were left on racks. The brine was able to run off. Afterwards the fillets were rinsed and chilled at 2°C until the next day. The normal cold smoking procedure used at the laboratory at DIFRES was applied. The fillets were dried for 2 hours at 26°C and smoked in an oven supplied with smoke generated from beechwood for 5½ hours at 26°C with a relative humidity of 75 %. After smoking the fillets were chilled at 2°C until next day.

The right fillets were cut into four pieces as the raw counterpart. For the smoked fillets, all pieces, except for the tail part, were each further divided into three smaller pieces (Figures 2.4 and 2.5). However for smoked rainbow trout, piece three was not divided into three smaller pieces. Each of these three smaller pieces was vacuum-packed and randomly assigned to one of three storage periods (1, 11 or 19/20) days at 2°C after smoking. After each storage period, analyses were performed. Only samples for chemical analysis were frozen. The temperature of 2°C was chosen due to practical reason as it was not possible to store the large number of samples in another chill storage room with a higher temperature.

**Raw fillet:**



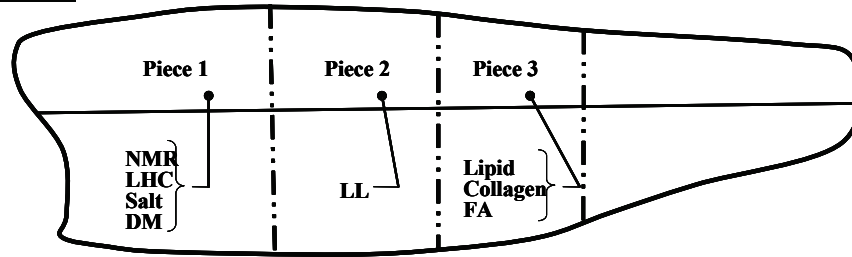
**Smoked fillet:**



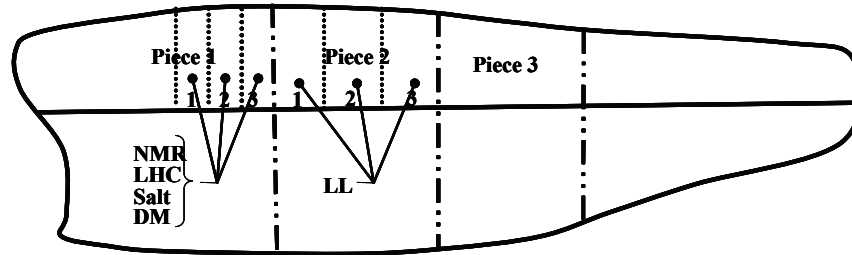
**Figure 2.4.** Sampling for experiment 2. The upper figure shows the sampling of raw samples and the lower figure shows the sampling of smoked samples. For the smoked fillet, pieces 1 to 3 were further divided into three parts. NMR is low-field NMR, LHC is liquid holding capacity measured by the centrifugation method, salt is salt content, DM is dry matter, LL is liquid loss measured by the liquid leakage test. Lipid is lipid content and collagen is collagen content. FA is measurement of fatty acid composition. Measurement of the salt content was only performed on the raw sample and on smoked fish one day after smoking.



**Raw fillet:**



**Smoked fillet:**



**Figure 2.5.** Sampling for experiment 3. The upper figure shows the sampling for raw samples and the lower figure shows the sampling of smoked samples. For the smoked fillet, pieces 1 and 2 were further divided into three parts. NMR is low-field NMR, LHC is liquid holding capacity measured by the centrifugation method, salt is salt content, DM is dry matter, LL is liquid loss measured by the liquid leakage test. Lipid is lipid content and collagen is collagen content. FA is measurement of fatty acid composition. Measurement of the salt content was only performed on the raw fish and on the smoked fish day 1 after smoking.

Four papers were made, and in table 2.2, it is shown which experiment belongs to what paper.

**Table 2.2.** Overview results used in Paper I to IV.

Paper	Results used
Paper I	NMR relaxation curves for raw and smoked salmon from experiment 2.
Paper II	A review paper on quality parameters in cold smoked salmon. Lipid contents from experiment 1 were used.
Paper III	Lipid contents, collagen contents, pH values and microscopically analysis of raw and smoked salmon from experiment 2 were used. The results from determination of liquid holding capacity (centrifugation method) for smoked salmon were also used. The water pools from experiment 2 (calculated in Paper I) for the smoked salmon were included in the multivariate data analysis to see effects of chill storage on smoked salmon.
Paper IV	Results from liquid leakage test and centrifugation method on raw and smoked salmon (experiment 2) and rainbow trout (experiment 3). The whole NMR relaxation curves for salmon and for rainbow trout were used to predict the liquid holding capacity.

## 2.2 Methodological considerations

In the following sections, the methods used for sensory evaluation of the raw material

---

Quality Index Method, measurements of salt, pH, lipid, fatty acid and collagen contents, measurements of liquid holding capacity and water distribution (NMR) are discussed. The microscopy techniques are briefly described. A short introduction to multivariate data analysis techniques used in this work is also given.

### **2.2.1 Quality Index Method (QIM) (experiments 1 and 2)**

The raw material quality in experiments 1 and 2 was evaluated by the Quality Index Method (QIM). QIM was originally developed by Bremner (1985) and has since been used and further developed in many European fisheries laboratories (Jonsdottir 1992; Martinsdóttir *et al.* 2001; Sveinsdóttir *et al.* 2002; Hyldig and Nielsen, 2004; Hyldig and Green-Petersen, 2005).

QIM is based on the characteristic changes that occur in raw fish during ice storage (Martinsdóttir *et al.* 2001; Hyldig and Green-Petersen, 2005). QIM schemes have been developed for various species and to take into account the differences between species it is necessary to develop a separate scheme for every species. A scheme with the most predominant descriptors for appearance, odour and texture is used and trained QIM assessors give a score from 0 to 3 for each of the descriptors. The Quality Index (QI) is obtained by summarising the scores for all parameters. The QI is zero for a very fresh fish and increases as the fish deteriorates. The QI increases with the keeping time in ice, so if the scheme has been constructed appropriately, the total sum of demerit scores can be used to predict the remaining shelf life (Martinsdóttir *et al.* 2001).

Table 2.3 shows the (QIM) scheme for farmed Atlantic salmon (Martinsdóttir *et al.* 2001). Trained assessors from an internal panel at the laboratory at DIFRES carried out the evaluations. The fish were marked with a number around the tail. In experiment 1, the QIM evaluation was performed at the commercial smoke house. The conditions were optimized as much as possible. The fish were placed on parchment paper on a table in the production hall and the evaluation was performed during a break for the workers. In experiment 2, the QIM evaluation was performed at the laboratory at DIFRES. The fish were placed in daylight and on cooled bricks during the evaluation in order to minimize quality changes. All assessors evaluated all the fish at the same time.

**Table 2.3.** The Quality Index Method (QIM) scheme used in the evaluation of raw farmed Atlantic salmon. Adapted from Martinsdóttir *et al.* (2001).

Quality parameter		Description	Score
Skin	Colour/ appearance	Pearl-shiny all over the skin	0
		The skin is less pearl-shiny	1
		The fish is yellowish, mainly near the abdomen	2
	Mucus	Clear, not clotted	0
		Milky, clotted	1
		Yellow and clotted	2
	Odour	Fresh seaweedy, neutral	0
		Cucumber, metal, hay	1
		Sour, dish cloth	2
		Rotten	3
	Texture	In rigor	0
		Finger mark disappears rapidly	1
Finger leaves mark over 3 seconds		2	
Eyes	Pupils	Clear and black, metal shiny	0
		Dark, grey	1
		Matt, grey	2
	Form	Convex	0
		Flat	1
		Sunken	2
Gills	Colour	Red/dark brown	0
		Pale red, pink/light brown	1
		Grey-brown, brown, grey, green	2
	Mucus	Transparent	0
		Milky, clotted	1
		Brown, clotted	2
	Odour	Fresh, seaweed	0
		Metal, cucumber	1
		Sour, mouldy	2
Rotten		3	
Abdomen	Blood in abdomen	Blood red/not present	0
		Blood more brown, yellowish	1
	Odour	Neutral	0
		Cucumber, melon	1
		Sour, fermenting	2
		Rotten/rotten cabbage	3
Quality Index			0-24

### 2.2.2 Determination of liquid holding capacity (experiments 1, 2 and 3)

The term liquid holding capacity for fatty fish refers to both water- and lipid-holding capacity i.e. the ability of a given structure to prevent water and/or lipid from being released from the structure (Hermansson, 1986).

---

In this work, three methods were used to determine liquid holding capacity. In experiment 1, a filter press method was used. In experiments 2 and 3 both a centrifugation method and a liquid leakage test were used. The centrifugation method and the filter press method are “active” methods which are based on the use of external forces e.g. centrifugation and compression. However, in the centrifugation method mince is used, while the filter press method uses intact muscle. The liquid leakage test is a “passive” method as it measures free drip, which refers to the amount of liquid lost without application of an external force.

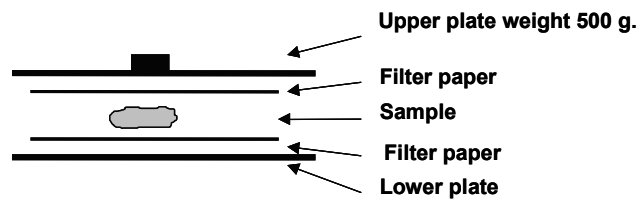
The filter press method does not require advanced equipment and can be applied at the production plant. However, high variations in liquid loss were observed for both raw and smoked samples. In addition, the filter press method is time-consuming, so it was decided not to use this method in the further experiments as a larger number of fish were to be analysed on the same day. Instead a centrifugation method and a liquid leakage test were used in experiments 2 and 3. Both methods were applied to the same sample to get a detailed picture as possible of the liquid holding capacity. The two methods measure different properties e.g. liquid loss from an intact muscle without an external force applied and liquid loss from minced muscle with an external force applied to the full sample.

In addition, it was also investigated if the liquid leakage test could substitute the centrifugation method which is only feasible in the laboratory. The liquid leakage test does not require advanced equipment during analysis as the centrifugation method requires and is similar to the drip loss method used in the industry. Low-field NMR curves were correlated to the values obtained by the centrifugation method and the liquid leakage test to see if the NMR signals could be related to the phenomena measured by these methods.

#### *The Filter press method*

The filter press method determines the amount of liquid that is squeezed out from an intact muscle piece during three minutes under the pressure of 500 g (Højmarklaboratoriet, 2005). Two pieces of Whatman GFA filter paper 12.5 cm in diameter were weighed ( $w_1$ ). The upper filter paper was moved. A thin piece of nylon cloth was placed on the other filter paper in order to prevent the sample from sticking to the filter paper. The scale was set at zero. A piece of untrimmed muscle (approx. 8-10 g) approx. 0.5 cm thick was cut

from the loin (over the lateral line) and the skin was removed. The piece was placed on the filter paper on the scale and weighed ( $w_s$ ). Another piece of nylon cloth was put on top of the sample, on top of which the upper filter paper was placed. The filter papers with the sample in between were placed on the plate. Another plate was placed on top of the filter papers (see Figure 2.6). The upper plate weighed 500 g. After 3 minutes the upper plate was removed and the sample was removed. The two filter papers were weighed again ( $w_2$ ). The filter papers were dried in an oven at 102°C for one hour. Afterwards, the filter papers were cooled down and weighed again ( $w_3$ ). The liquid loss (LL) was determined as  $LL (\%) = ((w_2 - w_1)/w_s) * 100$ .



**Figure 2.6.** Illustration of the filter press method. Adapted from Højmarklaboratoriet (2005).

#### *The liquid leakage test*

The liquid leakage test by Mørkøre *et al.* (2002) gives an indication of liquid loss of the material without deformation or destroying the microstructure of the sample during sampling. Intact fillets were placed on absorbing pads for 20 hours at room temperature and the liquid released was measured. The method by Mørkøre *et al.* (2002) was used with some minor modifications. In experiment 2, another kind of pad was used as it was not possible to obtain the pad recommended by Mørkøre *et al.* (2002). In experiment 3, Whatman filter paper was used instead of a pad and a Petri dish instead of wrapping in aluminium foil and the sample size was reduced to approx. 10 g due to the smaller size of the fish. The changes from experiment 2 to experiment 3 were done due to high variations found in the raw data from experiment 2. The piece under the dorsal fin was used for the liquid leakage test on both experiments 2 and 3. The lipid loss and the water loss in the liquid loss were also determined. The weight of the pad was recorded initially ( $w_a$ ), after 20 hours ( $w_b$ ) and after drying ( $w_c$ ). The liquid loss in % was calculated as  $100 * (w_b - w_a)/m$ , water fraction (of the liquid loss) as  $100 * (w_b - w_c)/m$ , and lipid fraction (of the liquid loss) as  $100 * (w_c - w_a)/m$ , where  $m$  is the weight of the muscle sample.

---

### *The centrifugation method*

The centrifugation method by Eide *et al.* (1982) was used in both experiment 2 and 3. The piece in front of the dorsal fin was used for analysis after mincing. The liquid holding capacity was determined by centrifugation of 2 g of minced sample for 5 minutes at 10°C and 1500g. The liquid holding capacity was calculated in two ways: as the amount of liquid left in the mince after centrifugation relative to the original amount of liquid (LHC<sub>1</sub>), or relative to the dry matter content (LHC<sub>2</sub>). For fatty fish, fat free dry matter content is used in the calculations of LHC<sub>1</sub> and LHC<sub>2</sub>.

$$\text{LHC1} = \frac{100 - \text{dm} - \Delta r}{100 - \text{dm}} * 100 \%$$

$$\text{LHC2} = \frac{100 - \text{dm} - \Delta r}{\text{dm}}$$

$$\Delta r = \frac{\text{g sample (before)} - \text{g sample (after)}}{\text{g sample (before)}} * 100$$

Dry matter is dm (%) in the sample before centrifugation. For fatty fish, fat free dry matter content is used in the calculations of LHC<sub>1</sub> and LHC<sub>2</sub>.

### **2.2.3 Determination of lipid content and fatty acid composition (experiments 1, 2 and 3)**

The lipid content was evaluated both in raw salmon and in smoked salmon (not in smoked rainbow trout samples in experiment 3). As the lipid content varies in the muscle, it is very important to specify the exact area of sampling when comparing results. In the present study, the area behind the dorsal fin, also called the Norwegian Quality Cut, was used for determination of lipid contents in all three experiments. The piece was minced before analysis. The lipid extraction method by Bligh and Dyer (1959) (extraction with chloroform, methanol and water) was used for determination of lipid contents. The fatty acids of the lipids in the Bligh and Dyer extract were determined by preparation of methyl

---

esters (AOCS Official Method Ce 2-66, 1998a) that were in turn analysed by gas chromatography (AOCS Official Method Ce 1-b-89, 1998b).

#### **2.2.4 Determination of collagen content (experiments 2 and 3)**

The hydroxyproline content was determined according to a colorimetric method described by the International Organization for Standardization (ISO, 1994). Sulphuric acid was used instead of perchloric acid to dissolve the colour reagent. To convert the amount of hydroxyproline to collagen in salmon muscle, a factor of 11.42 was used (Sato *et al.* 1991). The area behind the dorsal fin was used for the analysis, and it was minced before analysis.

#### **2.2.5 Determination of salt content (experiments 1, 2 and 3)**

The salt content was determined according to AOAC methods (AOAC Method 976.18 in combination with AOAC Method 937.07 and AOAC Method 971.27, 2000). The salt content was determined in raw fish and in smoked fish one day after smoking. The salt content was calculated in two ways as the amount of % salt (NaCl) in relation to the sample weight or as % salt (NaCl) in the water phase. In experiments 1 and 2, the piece behind the dorsal fin was used for determination of salt content. In experiment 3, the piece in front of the dorsal fin was used for determining salt content.

#### **2.2.6 Determination of pH (experiment 2)**

The measurement of pH was performed on the area behind the dorsal fin (Norwegian quality cut) in experiment 2. The piece was minced for 2 x 5 sec at 5°C in a Knifetec, 1095 Sample Mill (Foss Tecator, Sweden). The measurement of pH was performed with an Autocal pH meter (Metrohm, Denmark). The electrode was placed directly into the flesh.

#### **2.2.7 Determination of water distribution by low-field NMR (experiments 2 and 3)**

Nuclear magnetic resonance (NMR) is a spectroscopic technique based on the magnetic properties of atomic nuclei and is widely used as an analytical technique in many scientific fields as it is a rapid and non-destructive method. Low-field  $^1\text{H}$  NMR is an excellent tool for measuring the water distribution in various food products. Low-field  $^1\text{H}$  NMR measures the properties of protons and is therefore a direct technique for investigating the total quantity of water and the state of water and its interaction in the fish muscle. In this

---

study, low-field  $^1\text{H}$  NMR was used to determine the water distribution. In this section, a brief description of the low-field NMR theory will be given. For a more detailed description of the NMR theory the reader is referred to Farrar (1989), Field (1989), Deleanu and Paré (1997), Colquhoun (1993) and Ruan and Chen (1998).

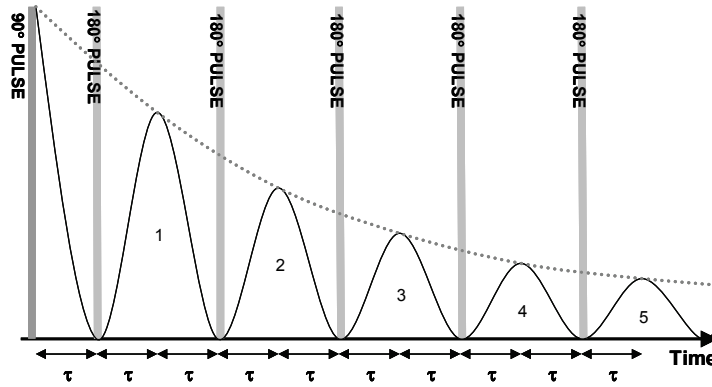
NMR spectroscopy is based on the absorption of energy through electromagnetic radiation by the nucleus with non-zero-spin in the presence of an externally applied magnetic field. The proton  $^1\text{H}$  is mainly used. In NMR, nuclei with a non-zero spin are active due to their magnetic moment or net spin of the whole sample. In the absence of a magnetic field, the non-zero spin nuclei are randomly oriented. When placed in an external magnetic field, the spins will align with the field or opposed to the field determined by the external magnetic field and the temperature. These two spin states have different energies. The NMR signal is produced by applying a suitable electromagnetic pulse whose energy is equal to the difference between the two spin states. The pulse tips the magnetisation away from the static field (Hemminga 1992; Deleanu and Paré 1997; Ruan and Chen, 1998). This induces an energy transition in the spins by the absorption of energy.

After application of a pulse, the spins will return to their equilibrium states at a certain rate. This decay rate is characterized by two relaxation time constants. These two constants are named spin-lattice relaxation time ( $T_1$ ) and spin-spin relaxation (or transverse relaxation time) ( $T_2$ ). The relaxations constants  $T_1$  and  $T_2$  can be measured by low-field NMR by applying different pulse sequences. The CPMG sequence developed by Carr and Purcell (1954) and Meiboom and Gill (1958) is often the preferred method for  $T_2$  measurements and was applied in the present study.

The CPMG sequence includes a series of pulses starting with a  $90^\circ$ -pulse followed by a number of  $180^\circ$ -pulses (Figure 2.7). After application of a  $90^\circ$ -pulse, all the spins are aligned in the plane perpendicular to the applied magnetic field. Following the pulse, the spins will de-phase at different rates due to inhomogeneity of the magnetic field and spin-spin interactions. After a short time denoted  $\tau$ , a  $180^\circ$ -pulse is applied. This reverses the direction of rotation and the spins now move (called re-phasing) back to phase. Application of a series of  $180^\circ$ -pulses spaced by a period of  $2\tau$  results in a train of echoes with



gradually decreasing intensity. According to common practice the relaxation curve is recorded as the maximum of every second echo (even echoes) as this corrects for imperfections of the pulse length (Farrar, 1989).



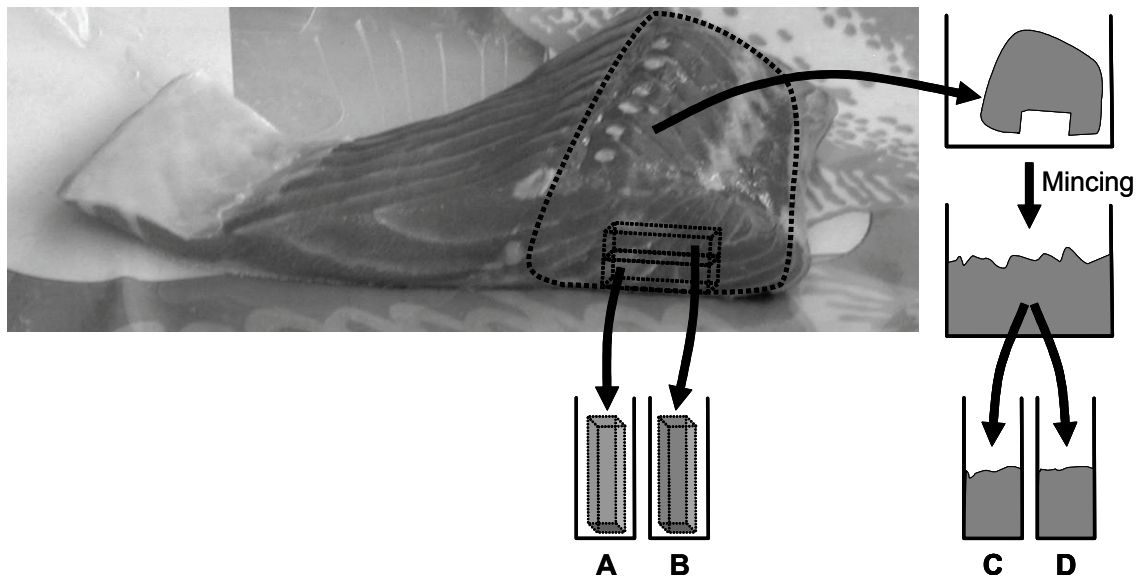
**Figure 2.7.** CPMG pulse sequence showing the first five echoes (solid line) and transverse relaxation curve (dashed line).

The low-field NMR relaxation signals obtained by the CPMG pulse sequence only measure liquid protons because the signal from solid-proton relaxation has decayed when the acquisition of data begins (Ruan and Chen, 1998). In fish muscle, this means that the measured relaxation is due to the presence of water and lipid. In fatty fish like salmon and trout, the majority of protons come from water, but a minor part will most likely be related to the storage lipid present in the muscle. The low-field NMR is performed at +8°C and some of the lipids will be in the solid state. The main part of the signal is therefore assumed to be water-proton relaxation.

The low-field NMR relaxation of a heterogeneous sample like fish muscle is composed of a sum of mono-exponentials, indicating that there are several groups of water molecules with different mobility (Jensen *et al.* 2002; Jensen *et al.* 2005; Andersen and Jørgensen, 2004). The number of exponential components can be characterized by their relaxation time constant ( $T_2$  values), and the components correspond to the water pools existing in the muscle tissue.

In this work, low-field  $^1\text{H}$  NMR was applied on both intact and minced muscle from both raw and smoked salmon and rainbow trout, respectively. The piece in front of the dorsal

fin was used for both measurements. First two muscle cubes (named intact samples), each of approximately 2 g were cut and weighed into small cylindrical glass tubes that fitted into 18 millimetre NMR sample tubes. The first muscle cube (called sample A) was cut about two millimetres from the skin, avoiding the dark muscle. The second muscle cube (called sample B) was cut out next to sample A closer to the middle of the fish (Figure 2.8). The rest of the piece was minced for 2 x 5 sec at 5°C in a Knifetec, 1095 Sample Mill (Foss Tecator, Sweden). Portions of 2 g mince were weighed into a small cylindrical glass tube that matched the inner diameter of the 18 millimetre NMR sample tube.



**Figure 2.8.** Sampling of intact and minced samples for NMR measurements. Adapted from Paper I.

Water distribution in the samples was calculated from the NMR signals by the use of multi-way data analysis. Using the slicing method (Pedersen *et al.* 2001), the two-dimensional NMR relaxation curves were transferred to three-dimensional data. Applying Parallel Factor Analysis (PARAFAC) to three-dimensional data, the sliced NMR relaxation data were decomposed uniquely to underlying mono-exponential curves, where each curve represents a water population. The slicing method and PARAFAC are described in section 2.2.9.5. It can be rather difficult to decide which samples should be included in the PARAFAC model because the samples should have common  $T_2$  values. If the samples included do not have common  $T_2$  values, it will be difficult to determine the

---

number of components and thereby the water distribution.

## **2.2.8 Microscopy (experiment 2)**

Microscopy is an excellent tool for studying food microstructure. In this work two microscopy techniques were applied i.e. light microscopy (LM) and confocal laser scanning microscopy (CLSM).

### **2.2.8.1 Light microscopy**

Light microscopy (LM) is a well developed and widely used technique for studying the microstructure and composition of food systems in relation to their physical properties and processing behaviour. Several different types of LM exist e.g. bright field, polarizing and fluorescence microscopy. In this work only bright field microscopy was used and LM will refer to bright field microscopy in the following. The principle is that light is sequentially transmitted through the condenser, the specimen, an objective lens, a second magnifying lens, the ocular or eyepiece prior to reach the eye. The resolution in LM is suitable for studying changes at the cellular level and it is possible to differentiate between e.g. muscle proteins, collagen and lipid through specific staining. Light microscopes are limited to 500x and 1000x magnification due to the physics of the light and a resolution of 0.2  $\mu\text{m}$ . In addition, high-resolution, good-quality images of the microstructure can only be obtained from thin sections of the sample (Brooker, 1995) as it should be sufficiently thin for light to pass through. The specimen needs to be stained to provide contrast.

### **2.2.8.2 Confocal laser scanning microscopy**

Confocal laser scanning microscopy (CLSM) represents a suitable alternative method to LM to evaluate food microstructure as it requires a minimum of sample preparation. In addition, CLSM does not depend on the transmission of light through the specimen and is thereby not dependent on the surface. Thicker sections may thus be used. In fatty fish, the lipid is easily spread over the surface during sample preparation making it difficult to analyze for lipid distribution. CLSM is therefore a suitable instrument for the analysis of lipid distribution in fatty fish.

CLSM uses a focused, scanning laser to illuminate a well-defined depth in the specimen and information from this focal point is projected onto a pinhole in front of a detector. It is

---

only the light from a defined focal plane in the specimen that reaches the detector and produces an image. The specimen is moved up and down relative to the focused laser light, and thereby large numbers of optical sections can be obtained. By collecting a number of images at different planes of the specimen and then superimposing them electronically, three-dimensional information can be obtained (Blonk and van Aalst, 1993; Brooker, 1995).

The term confocal microscopy is often assumed to be synonymous with confocal fluorescence microscopy because it is the most commonly used (Brooker, 1995). However, a wide range of alternative confocal techniques exist such as reflected bright field, differential phase contrast and differential interference contrast microscopy (Cogswell and Sheppard, 1990). In confocal fluorescence microscopy, images are produced by using the laser light to excite a selective fluorescent dye that has already been introduced or allowed to diffuse into the system. As alternative specific localization of chemical components can be achieved using antibodies, enzymes and ligands labelled with appropriate fluorescent dyes. If the lasers in the CLSM instrument produce light of two or more wavelengths, it is possible to obtain multiple images of the same field showing the distribution of different chemical components such as lipid and protein (Brooker, 1995).

#### **2.2.8.3 Preparation technique**

The same preparation technique, cryo-technique, was used for the preparation of specimens for both microscopes. For all samples, blocks of muscles were excised from the muscle part below the dorsal fin (one centimetre above the lateral line). They were embedded in tissue tex O. C. T. Compound and frozen in liquid nitrogen. The frozen specimens were stored at -80°C until sectioning. The samples were sectioned frozen at -26 to -28°C in a freezing microtome for transverse cuts. For LM, the sections were 10 µm thick. CLSM does not acquire thin sections as the light microscopy does, so sections were 20 µm thick. The sections were mounted on glass slides.

The advantage of using the cryo-technique is a reduced risk of tissue shrinkage, which can occur using chemical fixation methods. However, the disadvantage of using the cryo-technique is that the microstructure may be damaged due to the growth of ice crystals

---

during freezing.

#### **2.2.8.4 Staining**

The slides were stained using two different staining methods. The thin sections (10 µm) were stained with Orange G and Methyl blue according to Sigurgisladóttir *et al.* (2001) with the use of glycerol as mounting media. By this method muscle cells were stained blue and protein yellow.

The thicker sections (20 µm) were stained with Nile blue (0.1 %) (Nile blue A: 72480, Fluka Chemika) for three minutes. After rinsing with water, glycerol was used as mounting medium. Another mounting medium CITIFLUOR (glycerol/PBS solution, AF1) was also tried. There is a small amount of Nile red in Nile blue, and it is Nile red which gives the colour to lipids (Brooker, 1991). The lipids will get an intense yellow fluorescence due to Nile red. Nile blue and Nile red have been used in conjunction with CLSM to localise lipid in various food products including fat spreads, cheeses and chocolate (Brooker, 1991; Blonk and van Aalst, 1993; Auty *et al.* 2001).

#### **2.2.8.5 Microscopes**

The thin sections, stained for collagen and protein, were examined in a light microscope (Olympus BX51). The thicker sections stained for lipid was examined in a Leica confocal laser scanning microscope (CLSM) (TCS4d, Leica Laser Technik GmbH, Heidelberg, Germany). The microscope was equipped with an Argon/Krypton Laser. For Nile blue, an excitation wave length of 488 nm and DD 488/568 (double dichroic, reflects at 488 and 568 nm) filter was used.

#### **2.2.8.6 Samples**

In this work, only the salmon samples were analysed by the microscopy analysis. For the LM, one series (raw, smoked day 1, smoked day 11 and smoked day 20) from each of the four groups of salmon was cut on the freezing microtome. Several cubes were cut for each sample. For each sample, two to four specimens were stained and examined by the light microscope. About 5 to 10 images were obtained for each specimen. From two of the four groups of salmon, one series (raw, smoked day 1, smoked day 11 and smoked day 20) was stained and investigated by CLSM. For each sample one or two specimens were examined

---

and one to two images were obtained by CLSM.

### **2.2.9 Data analysis (two-way and three-way)**

Modern advanced technical instruments create enormous amounts of raw data (Martens and Martens, 2001) and as a result multivariate and multi-way data analysis has found an increasing applicability in food research.

Several multivariate methods exist e.g. Principal Component Analysis (PCA), Multiple Linear Regression, Principal Component Regression and Partial Least Squares Regression (PLSR), which can be applied to two-way data (Esbensen, 2000). In this work, PCA and PLSR have been used for the analysis of two-dimensional data. PCA is used to obtain an overview of data and to identify outliers. PLSR is used to relate design variables to measured variables or to predict liquid holding capacity from NMR relaxation curves.

Having data structures of three or higher dimensions, the two-way multivariate methods cannot be applied. In this work, the multi-way analysis, Parallel Factor Analysis (PARAFAC), was applied on three-way data. The main principles behind PCA, PLSR and PARAFAC are explained briefly.

#### **2.2.9.1 Principal Component Analysis (PCA)**

PCA is used to divide an  $X$  matrix into a structure part and a noise part. The purpose of the analysis is to find “hidden phenomena,” i.e. the underlying systematic variation in the data (Wold *et al.* 1987; Esbensen, 2000). The data matrix  $X$  has  $n$  objects and  $p$  variables. The objects can be observations, samples, experiments etc., while the  $p$  variables are for instance measurements within each experiment, attributes etc. (Esbensen, 2000).

Multivariate data can be thought of as a swarm of data points in a multivariate space. The essential principle in PCA is the extraction of principal components (PCs) from the data matrix. The first PC (PC 1) is placed through the multivariate data swarm in the direction describing the largest systematic variation in data. The second PC (PC 2) is orthogonal to the first PC and is placed in the direction describing the second largest variation in data. PC 3 will lie along the direction of the third largest variation in data and so on for PC 4, PC 5 etc. (Esbensen, 2000). This continues until all systematic information has been described.

---

The largest information in the data is described by the first component PC 1, but this is not necessarily the most important information. The principal components are axes in a new system of coordinates.

Several plots can be used to illustrate and interpret the result from a PCA. The most important plots are the score-plot and the loading plot (typically the first component versus the second component). The score-plot shows how the samples are distributed in relation to one another and can show groupings among the samples including outliers. The loading plots show the relation between variables and how much each variable contributes to each component. The score and loading plots can be compared and give information on how the variables are related to the sample pattern. Samples which have the same variable pattern are placed close to each other, whereas samples which are different are placed far from each other. A similar interpretation can be made of the loading plot. Variables situated close to each other describe some of the same underlying variation in data, whereas variables situated far from each other describe different phenomena.

PCA is a useful tool for outlier detection. Outliers are samples or measurements that deviate from the normal observations and will have an “incorrect” effect on the calculated model.

#### **2.2.9.2 Partial Least Squares Regression (PLSR)**

Partial least squares regression (PLSR) is used for modelling two data matrices, called  $X$  and  $Y$ .  $Y$  can consist of one variable or several variables (Esbensen, 2000). PLSR is used to extract the systematic variation in  $X$  that is related to the variation in  $Y$ . This makes it possible to interpret the structures within and between  $X$  and  $Y$  and the model obtained may be used for predicting  $Y$  from  $X$  in future samples (Martens and Næs, 1989; Martens and Martens, 2001).

Prior knowledge about the samples, e.g. storage time, season or different farms etc. may be used as indicator variables (design variables) and be included in the modelling. Each design parameter is converted to a binary variable given the values, 0 or 1 indicating whether the design parameter applies to the sample or not (Martens and Martens, 2001). In this way, a discriminate analysis can be applied, where  $Y$  consists of classes of samples

---

with different indicator variables and these are predicted from observed variables in  $X$ . In addition PLSR can be used to establish a calibration model from which a reference value can be predicted in future samples.

The score and loading plots from the PLSR models are to be interpreted in the same way as the ones from PCA.

### **2.2.9.3 Validation**

The overall purpose of validation is to test the applicability of the model on new, similar sets of data which have not been used in the development of the model. To ensure that the optimal number of components is used in the model, several validation methods exist. In the present work cross validation was used. Cross validation is the most commonly used validation method. Cross validation is performed by repeatedly taking out different subsets of samples from the model and using them instead as temporary local sets of test samples (Martens and Martens, 2001).

An essential aspect of validation is to determine the optimal number of components. This can be done by inspecting a plot of explained variance or a plot of residual variation versus the number of components because the optimal number of components to be included in the model is the number that gives the clearest break point in the plot. If there are too few components (an underfitted model), some systematic variation is lost in the residual. Using too many components (an overfitted model) there is a risk of drawing noise into the model (Esbensen, 2000).

### **2.2.9.4 Three-way data analysis**

In the present study, none of the data was three-dimensional in origin. However, the two-way low-field NMR data can be converted by the slicing method to become three-way in structure and thereby be analyzed by Parallel Factor Analysis (PARAFAC) (Pedersen *et al.* 2001; Pedersen *et al.* 2002; Engelsen and Bro, 2003). One of the advantages of using PARAFAC to decompose the NMR data is that a set of common  $T_2$  (spin-spin relaxation time) values is determined.

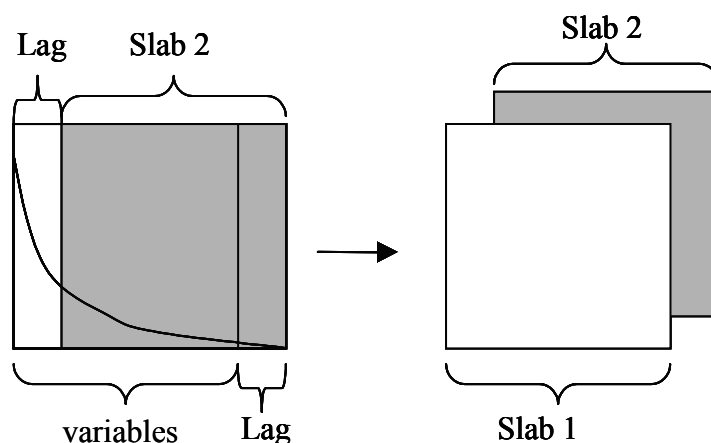
Slicing is based on the principal of Direct Exponential Curve Resolution (Windig and



Antalak, 1997) and can be used to analyse data consisting of a sum of exponentially decaying curves.

The three-way data structure can be created by copying the two-way data. The copy is placed behind the frontal slab, thereby creating a new third dimension. The back slab is shifted a specific number of variables to the right. The procedure is repeated. The two slabs contain the same number of variables. The two sub-matrices/slabs will be of the same size and contain the same information, but they are shifted horizontally. The number of variables to shift between two slabs is referred to as lag.

Slicing can be performed with more than two slabs and with different lag sizes. In Figure 2.9, only two slabs are shown for simplicity. A three-way structure can be obtained, when the splitting is performed in the same way for all samples in a data set.



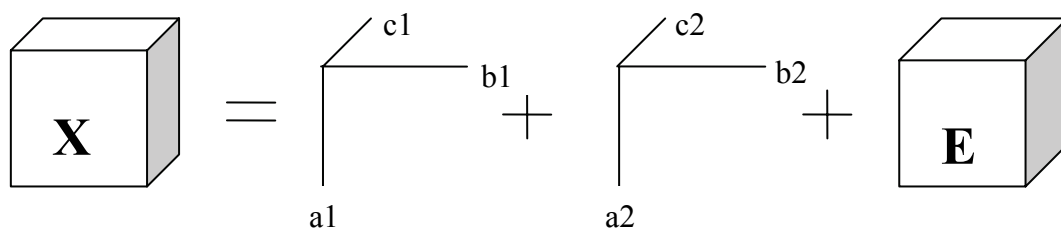
**Figure 2.9.** Illustration of the principle of slicing two-way NMR relaxation decays into three dimensional data. Lag represents the number of variables shifted in each layer and slabs represent the number of layers in the final three-way array.

Slicing in this direction generates curves of varying signal intensity but with the same underlying relaxation characteristics due to the unique mathematical properties of the exponential function (Windig and Antalok, 1997). This generates a tri-linear three-dimensional data array that can be decomposed by PARAFAC (Pedersen *et al.* 2002). Using slicing to generate three-way data from NMR relaxation decays requires several samples and the assumption that all the samples share the same underlying time constants

(Pedersen, 2001).

### 2.2.9.5 PARAFAC

Parallel Factor Analysis (PARAFAC) is a trilinear decomposition method for multi-way data, which can be seen as an extension of the two-way PCA (Harshman and Lundy, 1994). The method was proposed independently by Harshman (1970) and Carol and Chang (1970). The decomposition of three-way data using PARAFAC is illustrated in Figure 2.10.



**Figure 2.10.** An illustration of a two-component PARAFAC model of the data  $X$  after decomposing the original data matrix  $X$  into systematic variation and residual ( $E$ ). a, b and c represent the model vectors for each component in the PARAFAC model.  $E$  contains the variation which was not captured by the model. Adapted from Bro (1997).

PARAFAC of a three-way array  $X$  results in three matrices  $A$ ,  $B$  and  $C$  with three vectors  $a_i$ ,  $b_j$  and  $c_k$ , respectively.  $A$  is related to the samples ( $i$ ),  $B$  and  $C$  are related to the variables in the second ( $j$ ) and third ( $k$ ) dimension, respectively.  $A$  can be regarded as the scores, whereas  $B$  and  $C$  can be considered as the loadings generated for the two variable dimensions. In practice, it is not possible to distinguish between scores and loadings because these are treated equally numerically (Bro, 1997).

PARAFAC was developed to overcome the problem of “rotation freedom” in bilinear modelling (e.g. PCA). A PARAFAC model cannot be rotated without a loss of fit as opposed to a two-way analysis where the scores and loadings may be rotated without changing the fit of the model. The uniqueness means that there is only one solution that gives a certain fit.

A unique PARAFAC model revealing the underlying phenomenon requires that the

---

loadings are linearly independent, meaning that two loadings do not have the same shape. Additional requirements are that the right number of components is used, that the signal-to-noise ratio is appropriate and that data is trilinear (Harshman and Lundy, 1994; Bro, 1997).

A determination of the optimal number of components is essential in PARAFAC as it is in PCA and PLSR. There are three main ways of determining the correct number of components to be used; 1) split-half experiments, 2) by inspecting the residuals and 3) by comparing with external knowledge of the data being modelled (Bro, 1997).

The idea behind split-half is to divide the data into different subsets and then make a PARAFAC model on subsets. Due to the uniqueness of the PARAFAC model, the same result (same loadings) is obtained in the model of the subset of data if the correct number of components is chosen. An inspection of the residuals can also give an idea of the number of components. Prior knowledge of the data should be taken into account (Bro, 1997). In the case of NMR data, non-negative scores and mono-exponential loadings are expected in a PARAFAC model with the optimal number of components. From the logarithm of these loadings, the transverse spin-spin relaxation time ( $T_2$ ) can be determined and the size of each water pool is calculated. By applying PARAFAC, a set of common  $T_2$  values is determined for all samples, whereas the relative amounts of the various water pools differ.

---

## Chapter 3

### 3. Results and discussion

The results reported in Papers I to IV are presented and discussed in this chapter. Additional unpublished results are also included. In the first section, the quality of the raw material measured by QIM is described. In the second section, changes in the liquid holding capacity are described and the effects of salt content, lipid content and chill storage on the liquid holding capacity are also described. In the next section, the suitability of different methods to determine the liquid holding capacity is discussed. In the following sections, variations in salt content, lipid content, fatty acid composition and collagen content are described. In the next subsequent section, the water distribution in raw and smoked salmon muscle is described and it is shown how the distribution among the identified water pools is related to different parameters during chill storage. In the last section, changes in microstructure are described.

#### 3.1 Quality Index Method (QIM)

In experiments 1 and 2, the quality of the raw material was evaluated by the Quality Index Method (QIM). The total quality index (QI) was calculated as the total sum of demerit points given to each fish and an average was made of all assessments for each day of analysis. The total QI was compared to a QIM calibration curve in order to establish the relative freshness in terms of storage days on ice (Martinsdóttir *et al.* 2001). This gave an indication of how long the fish have been stored in ice before reaching the smoke house.

In experiment 1, the fish were analysed upon arrival (analysis day A) and after two or three days of chilled storage in ice at a commercial smoke house (analysis day B). On analysis day A, one box was analysed and on analysis day B, another box from the same batch as A was analysed. The analysis day (A) was 6-8 days after slaughtering according to the information given by the supplier, but the QI (from the analysis day A) indicated that the fish had been stored 9-14 days in ice. This indicates that the fish had not been cooled quickly after slaughtering or had not been stored at low temperature during transportation to the smoke house. In table 3.1, the QI for each day of analysis and the corresponding storage days in ice are shown for experiment 1.

**Table 3.1.** Quality Index (QI) averaged for each analysis day and the corresponding calculated days in ice (experiment 1). In week 4, the QI was only determined on day A.

Week	Day of analysis	Quality Index (average)	Days on ice
1	A	7	9
	B	10	13
2	A	11	14
	B	10	13
3	A	10	13
	B	12	16
4	A	11	14

A is the first analysis day and B is the second analysis day (2-3 days after A) in the same week.

In experiment 2, the fish were evaluated upon arrival at the laboratory at DIFRES. The QIM results showed that the fish had QI between 4 and 7, which corresponded to 5 to 7 days in ice before arrival. According to the information from the supplier, the fish were 5 and 6 days old upon arrival, i.e. little difference was observed, indicating that the fish had been stored at the correct temperature.

When the QI is above 20, the fish is no longer fit for human consumption. Thus, the maximum storage time of raw salmon is found to be 21 days under the best storage conditions (Sveinsdóttir *et al.* 2002; Sveinsdóttir *et al.* 2003).

QIM is a promising tool for a rapid and reliable assessment of the quality of the raw salmon and could be used at the smoke houses to assess the raw material upon arrival as the method measures the freshness of the fish.

### 3.2 Liquid holding capacity

The liquid holding capacity is an important quality parameter for cold smoked salmon as a reduced liquid holding capacity makes the fillets more prone to liquid leakage during smoking or afterwards during slicing and vacuum storage. Accumulated liquid leakage in vacuum-packaged smoked salmon has a negative effect on the product appearance (Birkeland, 2004).

---

Thus, it is important to have a high liquid holding capacity. In this work, three different methods were used to determine the liquid holding capacity of raw and smoked fish. As described in section 2.2.2, the filter press method was only used in experiment 1 as the method was found not to be a representative method to measure the liquid holding capacity in salmon due to high variations found in the results. In addition the filter press method is time-consuming and thereby not suitable when many samples are to be analysed. Therefore, it was decided to use other methods in the subsequent experiments. In experiments 2 and 3, both a centrifugation method and a liquid leakage test were used to determine the liquid holding capacity. These two methods measure different properties e.g. liquid loss from intact muscle without an external force applied and liquid loss from minced muscle with an external force applied to the full sample. It was decided to use both methods on the same fish to get a detailed picture as possible of the liquid holding property. Regarding the centrifugation method, the liquid holding capacity was calculated in two ways: as the amount of liquid left in the mince after centrifugation relative to the original amount of liquid ( $LHC_1$ ), and relative to the dry matter content ( $LHC_2$ ).

#### *Liquid holding capacity in raw and smoked samples*

In experiment 1, the raw salmon samples had a lower liquid holding capacity (mean liquid loss of  $2.4 \% \pm 0.5$ ) than the smoked samples (mean liquid loss of  $0.9 \% \pm 0.3$ ). For the raw samples, the liquid loss was in the range of 1.1 to 4.0 % and for the smoked samples in the range of 0.5 to 1.7 %. For both the raw and the smoked samples, a large variation in liquid loss was observed within each analysis day and for samples from the same batch. The raw material was very inhomogeneous with regards to lipid content and salt content and this may have influenced the liquid loss. Both salt and lipid content affect the liquid holding capacity as described in sections 1.5.

The liquid holding capacity measured by the centrifugation method showed that the raw samples in experiments 2 and 3 (both salmon and rainbow trout) had a higher liquid holding capacity ( $LHC_2$ ) than the smoked samples ( $p < 0.001$ ) had (Table 3.2) (Paper IV). A higher liquid holding capacity has also been observed for the raw samples compared to the smoked samples as reported by Birkeland *et al.* (2004a). The higher liquid holding capacity in the raw samples may due to a water loss during the processing. However, a higher liquid holding capacity in smoked samples compared to raw samples has been

reported by Rørå *et al.* (2003) and this was also observed in experiment 1. The difference observed in the results between experiment 1 and experiment 2 seems notable but might be due to the use of different methods and different sample conditions (intact muscle and mince) in the two experiments.

Regarding the liquid holding capacity relative to the original amount of liquid (LHC<sub>1</sub>), the raw salmon samples could be differentiated from the smoked salmon samples ( $p < 0.01$ ) stored for 20 days. The raw rainbow trout could also be differentiated from the smoked rainbow trout stored for a long time (19 days) ( $p < 0.05$ ). Both the rainbow trout and salmon (except from farm 1, large) had high liquid holding capacity (close to 100 %) (Table 3.2). Ofstad *et al.* (1993) also found that salmon had higher liquid holding capacity compared to cod. Thus, the ability to hold the liquid is high in salmon and rainbow trout.

**Table 3.2.** Liquid holding capacity in raw and smoked salmon and rainbow trout.

			Storage time after smoking			
Group			Raw	Day 1	Day 11	Day 19/20
Ex. 2 (salmon)	Farm 1 small (N = 7)	LHC <sub>1</sub>	95.6 ± 2.1	94 ± 2.6	94.6 ± 2.6	92.7 ± 3.7
		LHC <sub>2</sub>	3.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	2.2 ± 0.2
	Farm 2 small (N = 6)	LHC <sub>1</sub>	97.6 ± 0.6	95.9 ± 2.7	96.8 ± 3.0	93.7 ± 4.8
		LHC <sub>2</sub>	3.7 ± 0.1	2.5 ± 0.2	2.6 ± 0.1	2.3 ± 0.4
	Farm 1 large (N = 2)	LHC <sub>1</sub>	87.3 ± 1.0	85.6 ± 1.9	82.7 ± 0.4	80.8 ± 0.1
		LHC <sub>2</sub>	3.3 ± 0.4	2.5 ± 0.5	2.4 ± 0.4	2.0 ± 0.2
	Farm 2 large (N = 3)	LHC <sub>1</sub>	93.6 ± 5.5	89.0 ± 3.8	91.9 ± 2.2	89.9 ± 4.3
		LHC <sub>2</sub>	3.4 ± 0.3	2.1 ± 0.2	2.4 ± 0.1	2.3 ± 0.1
	Mature, fed restrictively (N = 8)	LHC <sub>1</sub>	97 ± 0.9	97.6 ± 1.9	97.3 ± 2.1	95.3 ± 2.5
		LHC <sub>2</sub>	3.6 ± 0.6	2.3 ± 0.3	2.3 ± 0.3	2.0 ± 0.5
Ex. 3 (trout)	Immature, fed restrictively (N = 8)	LHC <sub>1</sub>	96.2 ± 1.4	95.8 ± 2.2	93.1 ± 4.0	91.1 ± 5.2
		LHC <sub>2</sub>	3.0 ± 0.2	2.2 ± 0.1	2.0 ± 0.3	1.9 ± 0.3
	Mature/immature, fed normally (N = 8)	LHC <sub>1</sub>	95.5 ± 2.8	95.9 ± 2.8	95.7 ± 3.1	92.9 ± 5.5
		LHC <sub>2</sub>	3.1 ± 0.3	2.2 ± 0.2	2.1 ± 0.2	1.9 ± 0.3
	Mature, fed normally (N = 8)	LHC <sub>1</sub>	97.3 ± 1.0	98.7 ± 0.6	98.5 ± 1.5	96.8 ± 1.6
		LHC <sub>2</sub>	3.4 ± 0.3	2.3 ± 0.1	2.2 ± 0.3	2.3 ± 0.2

Values are means ± standard deviation and N is number of samples

LHC<sub>1</sub> = the amount of liquid left in the mince after centrifugation relative to the original amount of liquid

LHC<sub>2</sub> = the amount of liquid left in the mince after centrifugation relative to the dry matter content

In experiment 3, there were four groups of rainbow trout which differed according to

---

maturity state, feeding method (restrictive or normal) and strain (Table 2.1). Regarding the liquid holding capacity, none of the different parameters were observed to have a significant effect on the liquid holding capacity. The reason for this could be due to the high variation within each group. A high variation in the salt content (between 2.6 and 5.1 %) and the lipid content (4.1 and 11.2 %) was observed for the four groups of rainbow trout. Both parameters are known to affect the liquid holding capacity.

In experiments 2 and 3, the liquid holding capacity was also determined by a liquid leakage test (liquid loss). The raw rainbow trout samples had a higher liquid holding capacity (measured by a lower liquid loss) compared to the raw salmon ( $p < 0.0001$ ) (Table 3.3). Changes were made in the method from experiment 2 to experiment 3 as high variations were found in the raw data for salmon. In addition, the salmon were larger than the rainbow trout, which maybe also have influenced the results. For the salmon, it was found that the size of the fish influenced the liquid loss as large smoked fish lost significantly ( $p < 0.001$ ) more liquid than the smaller smoked fish in raw and smoked fish (day 1). After 11 days of storage this difference had gone.

The smoked fish (day 1) had a higher liquid holding capacity (measured by a lower liquid loss) than the raw salmon. Higher liquid holding capacity (and thereby lower liquid losses) in smoked samples compared to raw fish were also observed by Rørå *et al.* (2003) using the same method and this was also seen for the smoked salmon in experiment 1 using the filter press method.

For the rainbow trout in experiment 3, no differences were found among the four groups with respect to liquid loss as determined by the liquid leakage test, which was also observed using the centrifugation method.



**Table 3.3.** Liquid loss (%) determined by the liquid leakage test in raw and smoked salmon and rainbow trout.

		Storage time after smoking			
	Group	Raw	Day 1	Day 11	Day 19 /20
Ex. 2 (salmon)	Farm 1 small (N = 7)	2.8 ± 0.5	1.0 ± 0.3	1.6 ± 0.4	3.0 ± 0.9
	Farm 2 small (N = 6)	2.8 ± 0.5	1.0 ± 0.2	1.5 ± 0.4	1.7 ± 0.6
	Farm 1 large (N = 2)	3.7 ± 0.2	1.9 ± 0.3	1.5 ± 0.1	2.7 ± 0.4
	Farm 2 large (N = 3)	4.3 ± 0.2	1.7 ± 0.1	1.9 ± 0.5	3.6 ± 1.1
Ex. 3 (trout)	Mature, fed restrictively (N = 8)	2.0 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.3
	Immature, fed restrictively (N = 8)	1.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2
	Mature/Immature, fed normally (N = 8)	2.1 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.7 ± 0.3
	Mature, fed normally (N = 8)	2.3 ± 0.9	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.3

Values are means ± standard deviation

N is number of samples

The lipid fraction and the water fraction in the liquid loss (determined by the liquid leakage test), were calculated. During chill storage of the smoked salmon (experiment 2), an increase in the lipid fraction was observed from day 11 to day 20 ( $p < 0.01$ ), while no significantly difference in water fraction was observed during chill storage. This is in accordance with Rørå *et al.* (2003), where the lipid fraction of the liquid loss was increased after 15 days of chill storage, while the water fraction did not increase. During chill storage of the smoked rainbow trout no difference was found for the lipid fraction or the water fraction.

#### *Effect of salt on liquid holding capacity*

In experiment 1, it was found that the liquid loss correlated to the salt content ( $r = 0.47$ ,  $p < 0.01$ ) for the smoked samples. Hence, a higher salt content gave a lower liquid holding capacity which is opposite to what has been reported by Ofstad *et al.* (1995).

For the smoked salmon in experiment 2, the salt content influences  $LHC_1$  ( $r = 0.62$ ,  $p < 0.001$ ) and  $LHC_2$  ( $r = 0.38$ ,  $p < 0.01$ ). For the smoked rainbow trout samples in experiment 3, the salt content influences  $LHC_1$  ( $r = 0.51$ ,  $p < 0.0001$ ), but not  $LHC_2$ . Thus, a higher salt content led to an increased liquid holding capacity, which was also seen by Ofstad *et al.*

---

(1995), but the results from experiment 2 are opposite to the results found in experiment 1. Different methods i.e. a filter press method and a centrifugation method were used to determine the liquid holding capacity. Both the samples from experiments 1 and 2 were dry salted but different dry salting methods were used. In experiment 1, the fillets were dry salted according to the recipe at the commercial smoke house, where the fillets were salted one, two or four days. In experiment 2, an in-house dry salting method (at the laboratory at DIFRES) was used and the fillets were salted for up to 8 hours (large fish).

Regarding the liquid leakage test, the salt content did not affect the liquid loss for the smoked salmon. However, for the smoked rainbow trout samples, the salt content had a negative effect on the liquid loss ( $r = -0.35$ ,  $p < 0.0001$ ). Thus, a higher salt content gave a higher liquid holding capacity for raw rainbow trout which was also found by the centrifugation method.

#### *Effect of lipid content on the liquid holding capacity*

A high lipid content has been reported to lead to a decreased liquid holding capacity (Mørkøre *et al.* 2001; Birkeland *et al.* 2004a). Mørkøre *et al.* (2001) found that a high lipid content (between 14.5 and 21.8 %) gave a lower liquid holding capacity (determined by the centrifugation method of Gomez-Guillén *et al.* 2000) in smoked salmon fillets. Birkeland *et al.* (2004a) also found a lower liquid holding capacity (determined by the centrifugation method of Gomez-Guillén *et al.* 2000) for smoked salmon with a high lipid content ( $20.2 \pm 1.1$  %) as opposed to smoked salmon with a low lipid content ( $16 \pm 1.0$  %).

In experiment 1, no correlation between liquid loss and lipid content was found for the raw or for the smoked samples. In experiment 2, a low liquid holding capacity ( $LHC_1$ ) was correlated to a high lipid content in raw salmon ( $r = -0.71$ ,  $p < 0.0001$ ) and in smoked salmon ( $r = -0.71$ ,  $p < 0.0001$ ).  $LHC_1$  is calculated by dividing with the liquid (water and lipid) content. An in-directly relation to the lipid content is expected. No correlation between the lipid content and  $LHC_2$  was found for the raw or the smoked salmon samples.

For the rainbow trout samples, a negatively correlation between the lipid content and  $LHC_1$  was found for the raw ( $r = -0.44$ ,  $p < 0.05$ ) and for the smoked ( $r = -0.46$ ,  $p < 0.0001$ ) samples (unpublished results). As seen for the salmon, no correlation between the lipid

---

content and LHC<sub>2</sub> was found for the raw or the smoked rainbow trout samples.

With respect to the liquid loss as determined by the liquid leakage test, the lipid content was correlated to the liquid loss for the raw salmon samples in experiment 2 ( $r = 0.51$ ,  $p < 0.05$ ). No correlation was found between the lipid content and the liquid loss in the smoked salmon samples. For the rainbow trout samples in experiment 3, no correlation between the lipid content and the liquid loss was found for the raw samples or for the smoked samples.

An effect of the lipid content on the liquid loss determined by the liquid leakage method was seen for the raw salmon samples. A relation between the lipid content and LHC<sub>1</sub> was found, but this relation is estimated to be affected by the way LHC<sub>1</sub> is calculated.

#### *Effect of chill storage on the liquid holding capacity*

The shelf life of commercial smoked salmon depends on which country (market) the products are available. Shelf lives between 14 days and 6 weeks have been reported (Cardinal *et al.* 2004). In experiments 2 and 3, the cold smoked products were chill stored at 2°C for up to 19 and 20 days after smoking. A decrease in the liquid holding capacity (measured by the centrifugation method) was observed between day 11 and day 20 ( $p < 0.01$ ) (Paper III). This was also seen for the smoked rainbow trout samples (experiment 3), as a decrease in the liquid holding capacity was observed between day 11 and day 19 (LHC<sub>1</sub>:  $p < 0.05$ ; LHC<sub>2</sub>:  $p < 0.001$ ) (Paper IV).

Rørå *et al.* (2003) found that storage time (5 or 15 days) and storage temperature (4 to 14°C) strongly affected the liquid holding capacity of smoked salmon as the liquid holding capacity decreased when the storage temperature increased and also when the storage time increased. The decrease was more pronounced when the two conditions were combined, where a liquid loss of 4.4 % was found after 15 days of storage at 14°C. In experiment 2, two sizes of salmon from two farms were analysed. The small (size 3-4 kg) smoked salmon from both farms had a liquid loss (determined by the centrifugation method) of 5 % after one day of storage after smoking and 7 % after 20 days of storage after smoking. The large smoked salmon could be separated according to the two farms as the large fish from farm one had a liquid loss of 14 % after one day of storage after smoking and a liquid loss of 19 % after 20 days of storage after smoking, while the large smoked salmon from farm two

---

had a liquid loss of 9 % after one day of storage after smoking and 9 % after 20 days of storage after smoking (Paper III). The liquid losses found in the present study were higher than those found by Rørå *et al.* (2003). In the present study fish of size 3-4 kg and 6-7 kg were used, whereas Rørå *et al.* (2003) used fish of size 1-1.5 kg. Large fish lost more liquid and this might explain the differences between the studies. As the size of the fish influences the liquid loss, it is difficult to compare the results in order to see an effect of the different storage temperatures.

The effect of chill storage on the liquid loss measured by the liquid leakage test showed the same as seen for the centrifugation method. During chill storage at 2°C of the smoked salmon samples (experiment 2), a decrease in the liquid holding capacity (measured as an increase in the liquid loss) was observed between day 11 and day 20 after smoking ( $p < 0.001$ ) (Paper IV). Rørå *et al.* (2003) also used the liquid leakage test on smoked salmon and found that after 15 days of storage of the smoked samples, the liquid loss was at the same level as that observed for the raw fish. For smoked salmon (experiment 2), an increase in the liquid loss from 1.2 % on day 1 after smoking to 2.6 % on day 20 after smoking was observed during chill storage of the smoked product. Rørå *et al.* (2003) found liquid losses of 2.8 % (storage temperature of 4°C) and 6.3 % (storage temperature of 14°C) after 15 days of storage. Rørå *et al.* (2003) used smaller fish (size of 1-1.5 kg) than that used in experiment 2, but slightly higher liquid losses were found for a storage temperature of 4°C compared with the losses found in experiment 2, where a storage temperature of 2°C and a storage time of up to 20 days after smoking was used. Thus, there seems to be an effect of the storage temperature on the liquid loss when the results from Rørå *et al.* (2003) are compared with those obtained in experiment 2. But in experiment 2, the liquid leakage test by Mørkøre *et al.* (2001) was slightly modified and this may have influenced the results. For the rainbow trout (experiment 3), no changes in liquid losses were observed during chill storage of the smoked product (Paper IV).

From the results obtained in experiments 2 and 3, a reduced liquid holding capacity was observed in the smoked products after chill storage for nearly three weeks, whereas none or only very little visible liquid was observed in the vacuum packages containing smoked products showing that the decrease in liquid holding capacity did not result in severe liquid loss. A lower storage temperature (2°C) was used in the experiments 2 and 3 than

---

what is used in retail shops (5°C), which might have had an influence.

To conclude, the results from these experiments show that the liquid holding capacity is a complex property influenced by many factors. Higher liquid holding capacity was found in raw samples compared to smoked samples using a centrifugation method. However regarding the results from the liquid leakage test and the filter press method, a high liquid holding capacity was observed for the smoked samples 1 day after smoking compared to the raw samples. For raw salmon, it was found that high lipid content gave a high liquid loss. The liquid holding capacity was affected by the salt content and the size of the fish. Regarding the effect of the salt content on the liquid holding capacity different results were obtained from the different methods. Some of the reported differences may be due to the methods used to determine the liquid holding capacity. During chill storage of the smoked products, a decrease in the liquid holding capacity was observed.

### **3.3 Relationship between methods to determine liquid holding capacity**

Different studies of liquid holding capacity in salmon and rainbow trout have used different methods and different experimental conditions, and this makes it difficult to compare the results. The results from section 3.2 regarding liquid holding capacity showed that different results were obtained when different methods were applied to the same fish to measure the liquid holding capacity. There is no standard method for measuring the liquid holding capacity. However centrifugation methods have commonly been used to measure the liquid holding capacity. A suitable measurement of this property is therefore advantageous as a part of a production control. Therefore there is a need for better understanding of how the results from the different methods are related to each other.

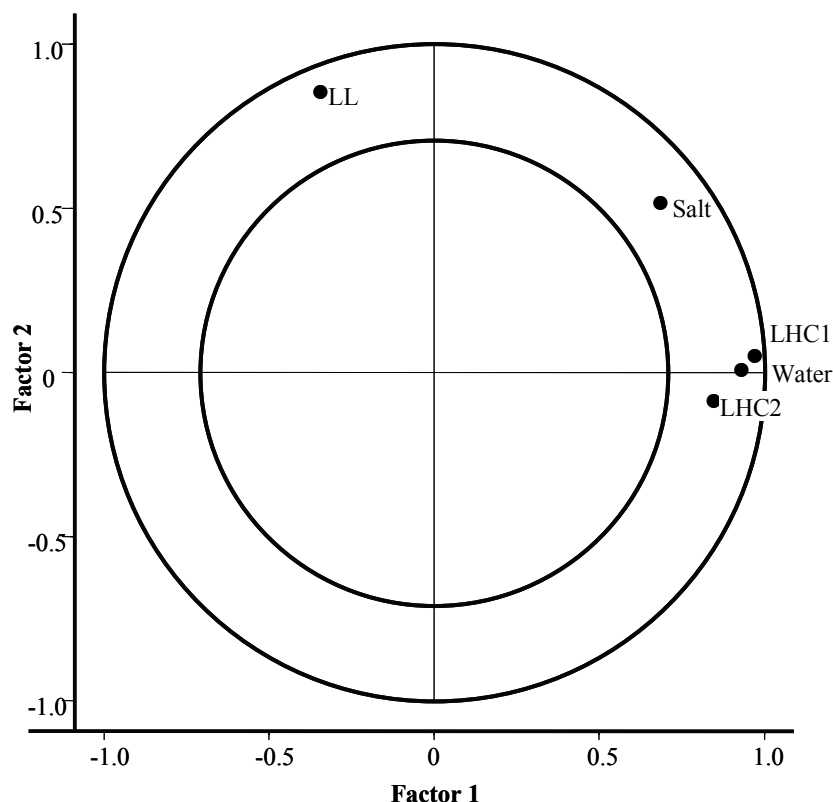
In Paper IV, a centrifugation method (Eide *et al.* 1982) and a liquid leakage test (Mørkøre *et al.* 2002) were examined with regard to their suitability for determining liquid holding capacity and liquid loss in salmon (experiment 2) and rainbow trout (experiment 3). Low-field <sup>1</sup>H NMR relaxation curves were correlated to values obtained by the centrifugation method and by the liquid leakage test to see if information in the NMR signals could be related to the phenomena measured by these two methods. The study included raw as well as smoked rainbow trout and salmon. All three methods were applied to the same fish. In experiment 1, a filter press method was used to determine the liquid holding capacity.

---

However this method was not included in the comparison of methods as it was not practically possible to include a fourth analysis on the same fish.

The liquid leakage test and the centrifugation method measure different properties (liquid loss from intact muscle without an external force applied and liquid loss from minced muscle with an external force applied to the full sample). Accordingly, the liquid leakage test and the centrifugation method were poorly correlated for smoked salmon ( $r = -0.32, p < 0.05$ ) and for smoked rainbow trout ( $LHC_1$ ):  $r = -0.25, p < 0.05$ ). No correlation was found between liquid loss and  $LHC_2$ . For raw rainbow trout samples (experiment 3), the liquid loss was negatively correlated to  $LHC_1$  ( $r = -0.40, p < 0.05$ ). For raw salmon samples (experiment 2), the correlation between liquid loss and liquid holding capacity was insignificant.

A Principal Component Analysis (PCA) was made of a data set containing LHC (both  $LHC_1$  and  $LHC_2$ ), liquid loss, salt and water contents of smoked salmon samples. The relationships between the variables are shown in Figure 3.1 as the so-called correlation loadings. Principal component 1 (Factor 1) mainly described the variation in LHC ( $LHC_1$  and  $LHC_2$ ) and water content, while Principal component 2 (Factor 2) mainly described the variation in liquid loss. The changes in liquid loss were almost independent of changes in the liquid holding capacity and the water content. Similar PCA results were obtained for smoked rainbow trout samples (results not shown).



**Figure 3.1.** Loading plot from a Principal Component Analysis (PCA) showing the relations between liquid loss (LL), LHC (LHC<sub>1</sub> and LHC<sub>2</sub>), salt and water contents (%) for smoked salmon samples. The inner and outer circles represent 50 % and 100 % explained variance, respectively. PC1 (factor 1) explained 46 % of X and PC 2 (factor 2) explained 15 % (validated). Adapted from Paper IV.

The low-field NMR relaxation curves were used to predict liquid holding capacity (both LHC<sub>1</sub> and LHC<sub>2</sub>) and liquid loss for minced and intact samples, respectively (Table 3.4), by Partial Least Squares Regression (PLSR). The PLSR models were evaluated by the correlation between the measured and the predicted values and by the Root Mean Squared Error of Prediction (RMSEP).

Prediction of the liquid holding capacity (both LHC<sub>1</sub> and LHC<sub>2</sub>) from the NMR relaxation curves worked well for the minced salmon samples and for the minced rainbow trout samples (Table 3.4). However, a tendency of non-linearity was observed for the prediction of LHC<sub>1</sub>. The correlation coefficients (correlation between measured and predicted LHC values) were close to that reported by Andersen and Jørgensen (2004) for cod (intact and minced) for the prediction of water holding capacity (LHC<sub>1</sub>) from NMR relaxation curves.

**Table 3.4.** Results from PLSR models for predicting e.g. liquid loss (LL) and liquid holding capacity (LHC) from NMR relaxation curves obtained for minced samples.

	Exp. 2			Exp. 3		
	LHC <sub>1</sub>	LHC <sub>2</sub>	LL	LHC <sub>1</sub>	LHC <sub>2</sub>	LL
No. of samples	70	70	70	128	128	127
No. of components <sup>a</sup>	2	2	3	4	4	4
Explained Y-variance <sup>a</sup>	71	78	33	64	86	82
RMSEP <sup>b</sup>	2.7	0.3	0.9	2.1	0.2	0.3
Correlation <sup>c</sup>	0.83	0.88	0.57	0.79	0.93	0.90

a: Optimal number of PLS-components and % variation regarding liquid loss or LHC explained by the validated model estimated by cross validation

b: Prediction error. Root mean square error of prediction

c: Model fit. Correlation between measured and predicted liquid loss or LHC values

LHC<sub>1</sub> = the amount of liquid left in the mince after centrifugation relative to the original amount of liquid

LHC<sub>2</sub> = the amount of liquid left in the mince after centrifugation relative to the dry matter content

For the intact samples, a good relation between NMR relaxation curves and LHC was also found (Table 3.5) with a correlation of 0.75 to 0.85 and RMSEP of 2.7 (LHC<sub>1</sub>) and 0.3 to 0.4 (LHC<sub>2</sub>).

**Table 3.5.** Results from PLSR models for predicting e.g. liquid loss (LL) and liquid holding capacity (LHC) from NMR relaxation curves obtained for intact samples.

	Exp. 2			Exp. 3		
	LHC <sub>1</sub>	LHC <sub>2</sub>	LL	LHC <sub>1</sub>	LHC <sub>2</sub>	LL
No. of samples	71	70	70	m <sup>d</sup>	63	63
No. of components <sup>a</sup>	2	3	3		4	4
Explained Y-variance <sup>a</sup>	70	53	22		74	76
RMSEP <sup>b</sup>	2.7	0.4	0.9		0.3	0.3
Correlation <sup>c</sup>	0.83	0.75	0.46		0.85	0.87

a: Optimal number of PLS-components and % variation regarding liquid loss or LHC explained by the validated model estimated by cross validation

b: Prediction error. Root mean square error of prediction

c: Model fit. Correlation between measured and predicted liquid loss or LHC values

d: No model calculated

LHC<sub>1</sub> = the amount of liquid left in the mince after centrifugation relative to the original amount of liquid

LHC<sub>2</sub> = the amount of liquid left in the mince after centrifugation relative to the dry matter content

Prediction of liquid loss from NMR relaxation curves for minced salmon and rainbow trout



---

samples is shown in Table 3.4 and for intact salmon and rainbow trout samples in Table 3.5. The prediction of liquid loss from NMR relaxation curves was better in prediction for rainbow trout (correlation of 0.90 and RMSEP = 0.3 for minced and a correlation of 0.87 and RMSEP = 0.3 for intact samples) than for salmon (correlation of 0.57 and RMSEP = 0.9 for minced and a correlation of 0.46 and RMSEP = 0.9 for intact samples).

The salmon had the highest lipid content and there appears to be a relation between higher lipid content and more lipid in the liquid loss: the lipid loss with time from salmon was higher relative to the water loss than was the case for trout (described in section 3.2). This may be one of the reasons why the prediction of liquid loss was better for rainbow trout than for salmon. In addition, changes in details of the liquid leakage test had been made from experiment 2 to experiment 3 to optimize the method, and this may also have influenced the results.

Models for prediction of liquid loss from the NMR data were made for raw and smoked samples separately (data not shown). These models were inferior to those including both raw and smoked samples indicating that liquid loss, unlike liquid holding capacity, is not closely related to the water distribution in the fish muscle but governed by other properties of the fillet. The information in the NMR signals used by the calibration with the full data set is rather about water content, which, like liquid loss, differs between the groups of raw and smoked fish.

It is important to be aware that the centrifugation method and the liquid leakage test measure different parameters and can therefore not substitute each other. Low-field NMR seems to be a promising tool for measuring the liquid holding capacity of salmon and rainbow trout samples. However further analysis is needed to recommend a suitable method for measuring liquid holding capacity in salmonid fish. Both the liquid leakage test and the centrifugation method depend on experimental conditions and require standardized conditions during analysis. The liquid leakage test has only been used in a few studies, and the disadvantage of the liquid leakage test is that numerous parameters such as the smoke hue, the size of the sample, etc (see Paper IV) can influence the results. The liquid leakage test has a potential as it does not require advanced equipment during analysis, while the centrifugation method is only feasible in a laboratory. In addition the liquid leakage test is

---

similar to the drip loss method used in the industry. Further investigations are needed before the method can be generally recommended.

To conclude, the various methods used for assessing liquid holding capacity focus on different aspects, making comparison of results difficult. Two of the most popular approaches, measuring the liquid holding capacity by centrifugation of minced muscle and measuring the liquid loss by the liquid leakage test, provide supplementary rather than redundant information. Thus, it is recommended to use both types of methods where applicable in order to get a detailed picture as possible of this important quality parameter. Low-field NMR relaxation curves were used to get some insight into the differences between the properties assessed by the centrifugation method and the liquid leakage test. A good prediction of liquid holding capacity from NMR relaxation curves measured on salmon and rainbow trout samples with liquid holding capacity values between 84-99% (LHC<sub>1</sub>) was obtained. The applicability of NMR relaxation curves to samples with lower liquid holding capacity values has not yet been tested.

### **3.4 Salt content**

In all three experiments, dry salting was applied as the salting method. The salt content was calculated in two ways as % salt (NaCl) in relation to the sample weight or as % salt (NaCl) in the water phase.

The salt content in smoked salmon is usually in the range of 2 to 4 % salt (Cardinal *et al.* 2004; Espe *et al.* 2004). In experiment 1, the salt content varied from 2.6 to 3.9 % salt (3.9 to 5.8 % salt in water phase) for salmon fillets salted for one day, 1.4 to 4.2 % salt (corresponding to 2.1 to 6.2 % salt in water phase) for salmon fillets salted for two days and between 1.8 to 5.5 % salt (corresponding to 2.7 to 8.1 % salt in the water phase) for salmon fillets salted for four days. The fillets were salted at the commercial smoke house according to their recipe. All the samples were salted in the same way (amount of salt and temperature (2°C) during salting), but the salting time was not fixed as the salting time varied from one to four days. This was the practice at the smoke house. However, no significant differences in the salt content were found between one day, two days or four days of salting. The fish were of the same size (3-4 kg) and the same amount of salt was used for each fillet side. Despite that, large variations in salt content were seen.

---

In experiments 2 and 3, the salting was performed at the laboratory at DIFRES according to an in-house salting recipe. The amount of salt used for salting was adjusted according to the size of the fillet and longer salting time was applied for the large fish. In experiment 2, the salt content was in range of 2.0 to 3.2 % salt (3.1 to 5.2 % salt in the water phase) for fish 3-4 kg in size. For the large fish (size 6-7 kg), the salt content was in the range of 2.4 to 3.2 % salt (3.8 to 5.1 % salt in water phase). In experiment 3, the salt content was in range of 2.4 to 5.1 % salt (4.1 to 7.7 % salt in water phase) for the smoked rainbow trout. Despite that the same salting method was used in both experiment 2 and 3, higher variation in salt content was found in experiment 3.

The diffusion of salt is an important issue during salting and the lipid content may be a limiting factor for the diffusion (Wang *et al.* 2000; Gallart-Jornet *et al.* 2007) either by replacing the aqueous phase that serves as a vector for transfer during the salting step or by acting as a physical barrier. Thus, the lipid content can affect the salt uptake. In experiment 1, the lipid content varied from 6 to 14 %, but no correlation between salt content and lipid content was found for the smoked salmon samples. In experiment 2, a correlation between the salt content and the lipid content in smoked salmon samples ( $r = -0.46$ ,  $p < 0.0001$ ) was found. In experiment 3, a correlation between the salt content and the lipid content in smoked rainbow trout samples ( $r = -0.48$ ,  $p < 0.0001$ ) was found.

To conclude, the salt content varied between 2 to 5 % (3 to 8 % salt in water phase) which is higher than the recommended value of 3.5 % salt in water phase. The lipid content may be a limiting factor for the diffusion of salt and it was found that a high lipid content gave a lower salt content.

### **3.5 Variation in pH**

In experiment 2, the pH of raw and smoked salmon was measured. The pH varied between 6.2 and 6.3 for the raw salmon and between 5.8 and 6.1 for the smoked salmon. Ofstad *et al.* (1995) found that pH greatly influenced the liquid holding properties in raw salmon as an increase in pH from 6.0 to 7.0 (in a model system) increased the liquid holding capacity. In experiment 2, the liquid holding capacity decreased for the smoked salmon during the chill storage period while the pH practically did not change in contrast to the result of

---

Ofstad *et al.* (1995). It should however be noted that the pH changes in the present study were on a much smaller scale than in the study by Ofstad *et al.* (1995). For the raw salmon samples, a correlation between pH and the liquid holding capacity (LHC<sub>2</sub>) ( $r = 0.47$ ,  $p < 0.05$ ) was found. Thus a higher pH value gave higher liquid holding capacity for raw salmon. No correlation was found for the smoked salmon samples.

To conclude, the pH was influenced by the process, but did not change during chill storage. For raw salmon, high pH gave high liquid holding capacity.

### **3.6 Variation in lipid content**

The lipid content is one of the quality characteristics of salmon that varies most, and high individual variation has also been observed (Bell *et al.* 1998). One of the major recent changes in cold smoking production has been the replacement of wild salmon by farmed salmon. Farmed salmon differs from the wild salmon by having on average a higher lipid content. The lipid content of salmon is usually in the range of 15 to 16 %, but it can vary from 6 to 22 % (Rørå *et al.* 1998).

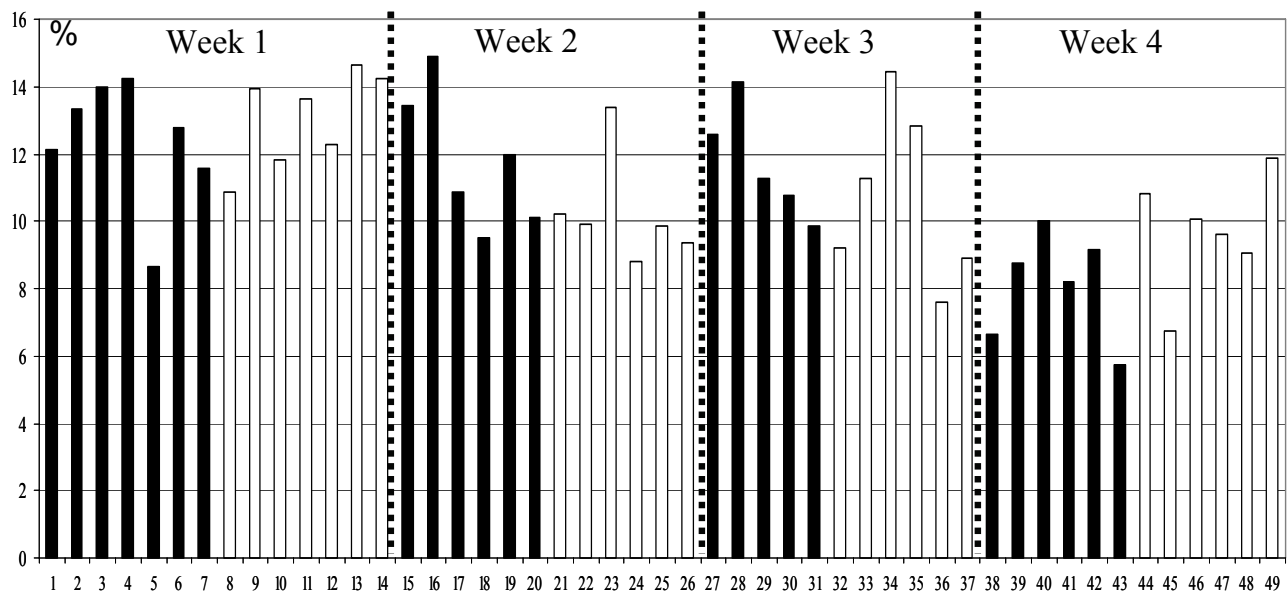
For salmon 3-4 kg in size, the average lipid content in the raw samples was 11 % (experiment 1, unpublished results) and 13 % (experiment 2, Paper III). In experiment 1, the lipid content in raw salmon varied between 6 and 14 %, whereas the variation in experiment 2 was much lower i.e. 11 % to 15 %. For the raw rainbow trout in experiment 3, lower lipid contents were observed compared to the raw salmon, as the lipid content varied between 4 and 11 % with an average of 7 % (unpublished results) (Table 3.6). For the smoked salmon samples, the lipid contents (not shown) were lower than for the raw samples, probably due to lipid loss during processing. The lipid content could be related to the body weight of the fish as the raw large salmon (size 6-7 kg) in experiment 2 had a higher lipid content than the smaller fish.

**Table 3.6.** Lipid content (%) in raw fish from all three experiments.

	No. of fish	Size kg	Lipid content (%)			
			Min	Max	Mean	SD <sup>a</sup>
Experiment 1	49	3-4	6	14	11	2
Experiment 2	13	3-4	11	15	13	1
Experiment 2	5	6-7	14	20	16	2
Experiment 3	32	2-3	4	11	7	2

a: standard deviation

In Figure 3.2, the variation in lipid content in raw salmon from experiment 1 is shown. In experiment 1, the lipid content within one box of raw salmon varied from 6 to 14 %, which illustrates a very heterogeneous raw material. Two boxes of salmon were analysed each week. The fish were analysed upon arrival (analysis day A coloured black in Figure 3.2) and after three days of chilled storage in ice at a commercial smoke house (analysis day B coloured white in Figure 3.2).



**Figure 3.2.** Lipid content (%) in raw salmon (black columns represent analysis day A and white columns represent analysis day B). On each day, one box of salmon was analysed.

Softness and gaping have been associated with high lipid content in farmed salmon (Sheehan *et al.* 1996), which can result in difficulties when slicing the smoked fish (Eckhoff *et al.* 1998). Birkeland *et al.* (2004a) found that fillets with a high lipid content were less firm and elastic than fillets with a low lipid content.

---

To conclude, in one experiment high variation in lipid content was observed for fish from the same batch of salmon, which indicate that the salmon have not been sorted with respect to lipid content at the supplier. In another experiment the fish had more equal content of lipid. Large salmon had a higher lipid content compared to small fish.

### **3.7 Variation in fatty acid composition**

Fish is a health-promoting food due to the high contents of n-3 polyunsaturated fatty acids (PUFA) (Kris-Etherton *et al.* 2002). The long chain n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) in fish are recognized to contribute to the positive effect of fish, particular fatty fish such as salmon have a high natural content of these fatty acids.

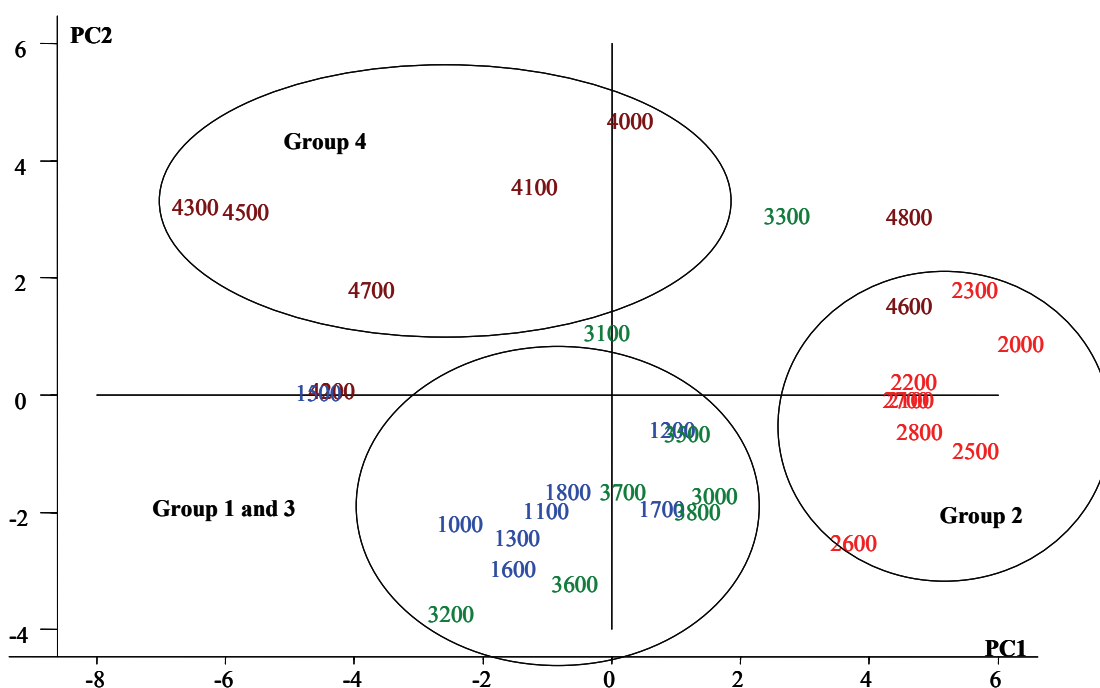
Fish meal and fish oil have traditionally been used as the major raw material ingredients in salmon feeds, and a high amount of the total global fish oil is used for fish feed production. However, the increase in the production of farmed fish has led to a reduced availability of fish oil and to an increased price of fish oil. Alternative oils are thus partially replacing fish oil in the diet of farmed salmonids (Sargent *et al.* 2001; Bell *et al.* 2001; Bell *et al.* 2002). Alternative oil sources are soybean, rapeseed and sunflower oil.

In all three experiments, the fatty acid composition of the lipids in the Bligh and Dyer extract were determined. Differences in fatty acid composition were observed between the samples mainly with respect to the contents of EPA and DHA. The differences observed in fatty acid composition might be explained by differences in the composition of the feed given to the fish because the fatty acid composition in the feed will be reflected in the fatty acid composition in the fish muscle (Hardy *et al.* 1987; Bell *et al.* 2001; Rørå *et al.* 2003; Torstensen *et al.* 2005). The fatty acid profile of the muscle does not change during the smoking (Rørå *et al.* 2003; Regost *et al.* 2004). Thus, the fatty acid profile of the raw material seems to be reflected in the smoked fillets.

In experiment 1, the fish from week 1 had higher contents of EPA ( $p < 0.0001$ ) and DHA ( $p < 0.0001$ ) than fish from week 2-4. The latter had higher contents of e.g. oleic acid and linoleic acid than the fish from the first week. The fish from week 1 were from one farm

and the fish in weeks 2-4 were from another farm. Thus, the differences observed in fatty acid composition may be related to differences in the fatty acid composition of the feed.

In experiment 2, fish from the two farms could also be separated according to their fatty acid composition e.g. contents of EPA and DHA (unpublished results). In experiment 3, the rainbow trout could be separated with respect to the fatty acid composition. Three of the groups were from the same farm and had been given the same feed, and they could be separated from fish from the other farm (group 4). However, for fish from the same farm (groups 1 to 3) variation was also observed, as the group (group 2) consisting of immature fish separated from the two other groups which were a group of mature and a group consisting of a mixture of mature and immature fish (Figure 3.3). Thus, the maturity may influence the fatty acid composition. However further investigation should be done.



**Figure 3.3.** PC 2 versus PC 1 scores from a PCA model with segmented cross validation (10 samples per segment) and auto scaled data. The samples are marked according to groups; group 1 (blue), group 2 (red), group 3 (green) and group 4 (purple). PC1 (factor 1) explained 28 % of X and PC 2 (factor 2) explained 9 %.

The effect of fatty acid composition on the liquid holding capacity has been studied, and contradictory results have been found according to the effect of fatty acids on liquid holding capacity. Rørå *et al.* (2005b) found a significant effect of dietary treatment on smoked fillets as the fish fed a diet with soybean oil had a lower liquid holding capacity

---

than fish fed a diet with fish oil. However another study, Rørå *et al.* (2003), found no effect of dietary oil sources on the liquid holding capacity in fresh and smoked salmon. In the present study, no effect of difference in fatty acid profile on the liquid holding capacity was observed.

The effect of using alternative oils in the feed for farmed salmon has been investigated in several studies. Alternative lipid sources can replace part of the fish oil in the diet without having a negative effect on growth (Bell *et al.* 2001; *et al.* 2002; Grisdale-Helland *et al.* 2002; Rørå *et al.* 2003). The effects of vegetable oil on pigmentation, texture, storage stability and sensory characteristics depend on the level of substitutions. In recently published studies (Bjerkeng *et al.* 1997; Rørå *et al.* 2003; Torstensen *et al.* 2005), it was shown that vegetable oil (either pure soybean oil or a mixture of rapeseed, linseed and palm oil) can be used in the grow-out phase without changing the quality in terms of texture and liquid holding capacity. However, a total substitution of the fish oil by soybean oil in diets for salmon affected the muscle colour (Rørå *et al.* 2005a).

To conclude, the fatty acid composition varied between fish from different farms, and this indicates that different feed combinations were used. No effect of the fatty acid composition on the liquid holding capacity was found.

### **3.8 Variation in total collagen content**

The total collagen content (g per 100g wet fish muscle weight) was determined for raw and smoked fish in experiment 2 (Paper III) and for raw fish in experiment 3 (unpublished results). The total collagen content varied between 0.38 to 0.52 g/100g with an average of 0.45 g/100g for raw salmon in experiment 2. This agrees with other studies of total collagen contents in Atlantic salmon muscle, where contents between 0.24 and 0.66 g/100g (Eckhoff *et al.* 1998; Aidos *et al.* 1999; Espe *et al.* 2004) have been found. For the smoked salmon (experiment 2), the total collagen content varied between 0.40 and 0.61 g/100g with an average of 0.50 g/100g. In experiment 2, the raw salmon samples had lower total collagen than the smoked salmon ( $p < 0.001$ ). During chill storage of the smoked salmon, an increase in the total collagen content was observed from day 1 to day 20 ( $p < 0.05$ ). During the process the fish loss water and thereby the dry matter content increases. Therefore, the increase in total collagen content is expected due to loss of water/moisture



---

during processing and chill storage.

For the raw rainbow trout in experiment 3, the total collagen content varied between 0.18 and 0.50 g/100g with an average of 0.32 g/100g. A larger variation in the total collagen contents was observed for the rainbow trout compared to the salmon. The rainbow trout samples were grouped into four groups. Groups 2 and 3 consisted of immature rainbow trout and a mix of mature/immature rainbow trout, and these two groups had significantly lower ( $p < 0.0001$ ) total collagen content compared to the groups of mature rainbow trout (unpublished results). The results are in accordance with the results obtained by Aknes *et al.* (1986), who found that the content of hydroxyproline and thereby the total collagen content was lower in immature than in mature salmon. Aknes *et al.* (1986) related the increase in hydroxyproline in mature salmon to the metabolization of protein from the muscle during maturation at the expense of non-collagenous and cellular protein (Love, 1970).

A high collagen content has been associated with firmer texture and less incidence of gaping (Sato *et al.* 1986; Hatae *et al.* 1986). Birkeland *et al.* (2004a) found that fillets with high lipid content were less firm and less elastic than fillets with a low lipid content. Therefore it was investigated if there was a relation between lipid content and total collagen content. In experiment 2, the lipid content was correlated to the total collagen content ( $r = -0.35$ ,  $p < 0.01$ ) for smoked salmon. Thus, high lipid content was related to low total collagen for smoked salmon. No significant correlation was found between lipid content and total collagen content for the raw salmon samples (experiment 2) or for the raw rainbow trout samples (experiment 3). To investigate if the relation between lipid content and collagen content were a result of variation in the dry matter content, the collagen content were calculated by fat free dry matter (referred to as collagen g per 100 g fat free dry matter). No correlation was found between the total collagen content g per 100 g fat free dry matter and lipid content in experiment 2 or 3. The correlation found between the total collagen (g per 100 g fish) and the lipid content in smoked salmon was probably due to differences in the dry matter contents.

To conclude, salmon and rainbow trout have a natural variation in total collagen. It was found that the state of maturity of the fish affected the total collagen content as mature fish

---

had higher total collagen content than immature fish. In this study, the results indicate no relation between the lipid content on the collagen content.

### **3.9 Water distribution**

This section presents the results from three-way decomposition of the low-field NMR relaxation measurements. The term water distribution refers to the relative sizes of the identified water pools.

In experiments 2 and 3, low-field NMR measurements were performed on minced and intact samples of salmon and rainbow trout. The NMR relaxation curves for both rainbow trout and salmon were used in Paper IV to predict the liquid holding capacity of rainbow trout and salmon. The results are discussed in section 3.3 and will not be discussed in this section.

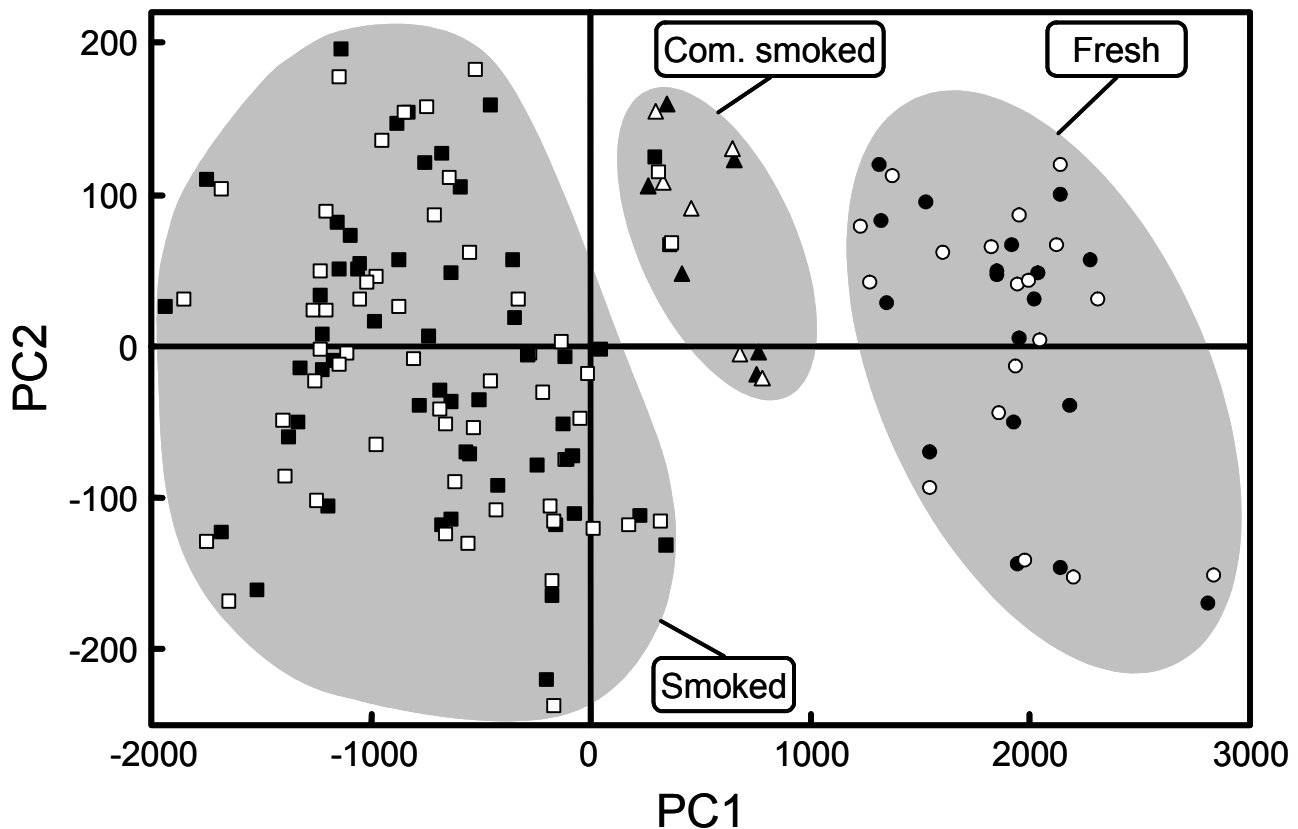
The first task in modelling NMR data is to determine whether all samples share the same underlying structure and then to determine the number of components, i.e. the number of common water pools in the samples. For the rainbow trout samples (experiment 3), it turned out that not all samples had a common underlying structure and it was not possible to model the data to determine the components although several attempts was made to arrange different groupings of the samples.

For minced and intact salmon samples (experiment 2), the water pool sizes were determined from low-field NMR relaxation signals, and these results will be discussed in this section.

Low-field NMR measurements were performed on both minced and intact salmon samples (both raw and smoked). The samples were arranged into four groups of salmon, small and large fish from two different farms. These samples are referred to as in-house smoked samples. Three samples from the group consisting of small salmon from farm two were dry salted and smoked at a commercial smoke house, and they are referred to as commercial samples. After smoking, the commercial samples were sent to the laboratory at DIFRES and they were cut into the same pieces as the in-house smoked samples. Analysis of the commercial samples was performed on day 6 and day 15 after smoking. Commercial

samples were included in both data sets (mince and intact).

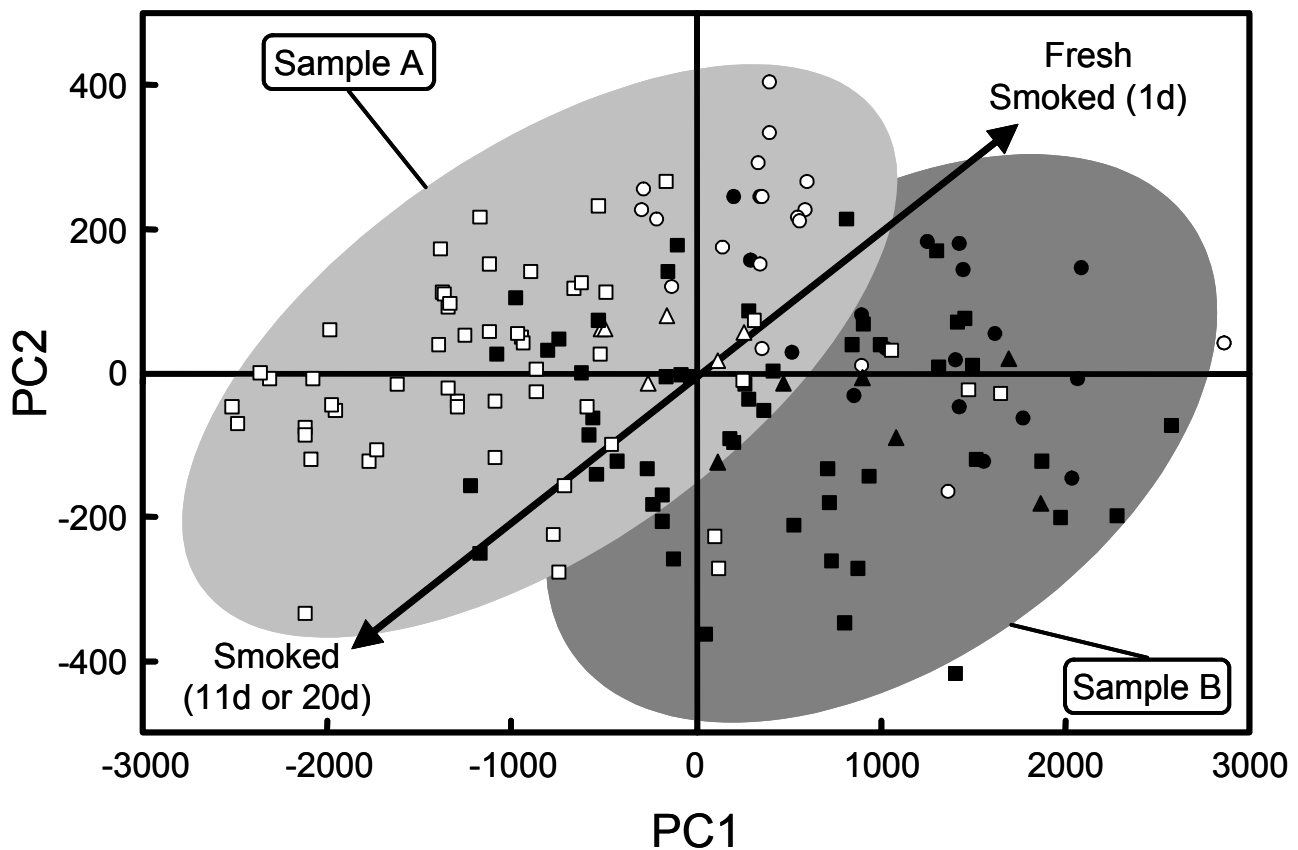
Before calculating the water pools, the NMR relaxation curves were inspected by PCA models. For the minced samples, an effect of the smoking process was observed as the first principal component differentiated between raw and smoked salmon (Figure 3.4). In addition, a third group of commercially smoked samples lying between the raw and the smoked samples could be identified. These commercially smoked salmon were from the same batch as the small fish from farm two. However, the cold smoking process (salting and smoking) was different and had apparently affected the water distribution.



**Figure 3.4.** Component 2 versus component 1 scores from a PCA model on NMR relaxation profiles of raw (fresh) and smoked minced salmon samples. Raw replicate C (●) and D (○); commercially smoked replicate C (▲) and D (△); in-house smoked replicate C (■) and D (□). Adapted from Paper I.

For the intact samples (Figure 3.5), another aspect was observed, as the samples tended to separate according to sample A and sample B. This is probably due to the heterogeneity of the salmon fillets. The samples were always cut side by side, but sample A was cut approximately two millimetres from the skin (avoiding dark muscle in the sample), whereas sample B was always cut nearest to the middle of the fillet. This sampling may have led to different chemical compositions of the two samples as the lipid content is

usually higher (and the water content lower) near the skin (Mannan *et al.* 1961). Thus, an explanation of the sample pattern, i.e. different water distribution between samples A and B, seen in Figure 3.5, could be a difference in the chemical composition with sample A having a higher lipid content than sample B. Refsgaard *et al.* (1998) showed that the distribution of lipid varied throughout the salmon fillet, which illustrated a lack of uniformity of the fillet. The present results clearly show the effects of sampling, and that even small distances between two close sampling points can result in marked differences.



**Figure 3.5.** Component 2 versus component 1 scores from a PCA model on NMR relaxation profiles of raw and smoked intact muscle samples from salmon. Raw replicate A (●) and B (○); commercially smoked replicate A (▲) and B (△); in-house smoked replicate A (■) and B (□). The points are also systematically distributed according to storage time after smoking as indicated by the arrows. Adapted from Paper I.

In addition (Figure 3.5), the pattern was somewhat different for the intact samples than for the minced samples as the raw and the commercially smoked samples did not form separate groups. A group consisting of raw samples, smoked samples 1 day of storage and commercially smoked samples separated from the smoked salmon stored for longer time (11 or 20 days).

Three water pools were characterised by their transverse relaxation times ( $T_2$ ) of 33 ms (pool I), 50 ms (pool II) and 183 ms (pool III) for minced samples. For the intact samples, the transverse relaxation times ( $T_2$ ) were 38 ms (pool I), 50 ms (pool II) and 182 ms (pool III) (Table 3.7). Water pool III represented the most mobile water, while water pool I was the most tightly bounded water. In a study by Jepsen *et al.* (1999)  $T_2$  values of 39, 84 and 353 ms were obtained for fresh, minced salmon after exponential fitting. In the present study,  $T_2$  values of 33, 50 and 183 ms were found for minced (raw and smoked) salmon by using PARAFAC modelling. The differences between the results of the two studies may be due to variations in the sample preparation, lipid content and the method of data analysis. In addition the present study also included smoked salmon.

The low-field NMR relaxation signals obtained by the CPMG pulse sequence only measure liquid protons because the signal from solid-proton relaxation has decayed when the acquisition of data begins (Ruan and Chen, 1998). In fish muscle, this means that the measured relaxation is due to the presence of water and lipid. In fatty fish such as salmon and rainbow trout, the majority of protons come from water, but a minor part will most likely be related to the lipid present in the muscle. The low-field NMR was performed at +8°C and some of the lipids will be in the solid state. The main part of the signal is therefore assumed to be water-proton relaxation. However there might also be a contribution from lipid protons.

**Table 3.7.** Relaxation times of water pools in minced and intact samples from raw and smoked salmon.

Sample set	Pool I $T_2$ (ms)	Pool II $T_2$ (ms)	Pool III $T_2$ (ms)
Intact muscle	38	50	182
Minced	33	50	183

The size of each water pool was calculated. The relative water pool sizes in minced salmon samples are shown in Table 3.8. The relative water distribution in the minced salmon samples revealed that the size of water pool II was significantly higher and water pool I was significantly lower in the raw samples compared with the in-house smoked samples ( $p < 0.0001$ ). The commercial samples also had a significantly higher content of water in pool II and a significantly lower content of water pool I compared to the in-house smoked

sample ( $p < 0.0001$ ), but they were also significantly different from the raw samples. The results indicate that the water distribution is affected by the cold smoking process. For the in-house smoked samples, a decrease in the size of water pool II and a parallel increase in the size of water pool I were seen during the chill storage from day 1 to day 20 after smoking with the greatest change happening between 1 day and 11 days of storage after smoking. A similar change in water distribution was also observed during chill storage of thawed cod packed in a modified atmosphere, where an increase in the size of water pool II at the expense of pool I was found to correspond to the denaturation of muscle protein and a simultaneous change in pH (Jensen and Jørgensen, 2003).

**Table 3.8** Relative water pool sizes in minced salmon samples (Adapted from Paper I).

	Number of samples	Pool I		Pool II		Pool III	
		Mean	SD	Mean	SD	Mean	SD
Raw	32	0.12	0.07	0.82	0.07	0.06	0.01
Day 1 <sup>a</sup>	36	0.51	0.09	0.44	0.09	0.05	0.01
Day 11 <sup>a</sup>	36	0.60	0.08	0.36	0.08	0.05	0.01
Day 20 <sup>a</sup>	35	0.64	0.09	0.31	0.09	0.06	0.01
Commercial	12	0.33	0.05	0.62	0.05	0.05	0.01

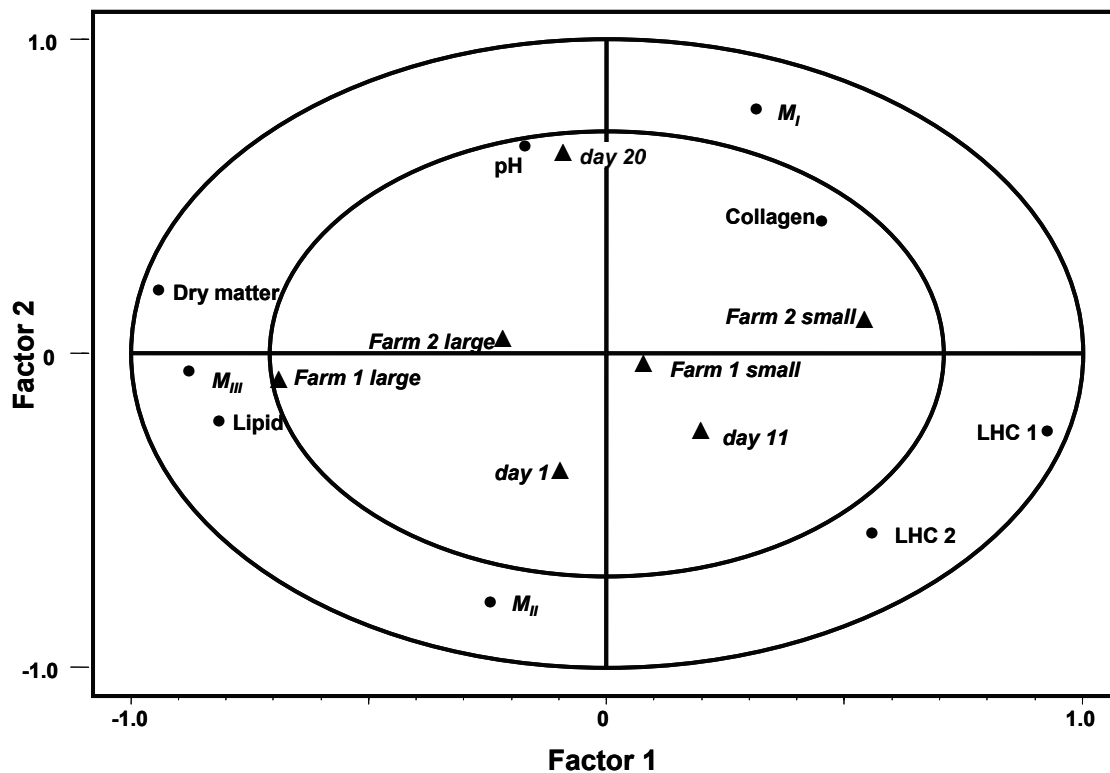
a: in-house smoked samples

The sizes of water pools I and II in the commercial samples were between the values for raw samples and in-house smoked samples (day 1), even though the commercial samples had been stored for up to 15 days. The commercial samples had been through a different salting and smoking process, which included longer salting at lower temperature and a longer smoking time. The smoking was carried out in a traditional oven, where the temperature was slowly increased. This smoking procedure may be more gentle resulting in less denaturation of proteins compared to the in-house smoked salmon. The differences between the products are reflected in the water distribution.

For the intact samples, the three water pools identified were identical for the A and B samples, while the water distribution was different between the A and B samples. The intact A samples had a higher content of water in pool II, while the intact B samples had a

higher content of water in pool I ( $p < 0.0001$ ).

A PLSR model was made for intact smoked B salmon samples with the  $X$ -matrix consisting of response variables (chemical and functional properties) and the  $Y$ -matrix consisting of indicator variables (values either 0 or 1) for the four groups and three storage times after smoking. The correlation loadings for the first two components from the model of intact smoked B samples are shown in Figure 3.6



**Figure 3.6.** Correlation loadings with factor 2 versus factor 1 from a PLSR model relating the response variable  $X$  (●) and design  $Y$  variable (▲) for intact smoked B salmon samples. Segmented cross validation was used and five components explained 21.2 % of the  $y$  validated variation. Days 1, 11 and 20 are days of storage after smoking.  $M_I$ ,  $M_{II}$  and  $M_{III}$  are the relative sizes of water pools I, II and III, respectively. Collagen is  $\text{g kg}^{-1}$ .  $\text{LHC}_1$  is the amount of liquid left in the mince related to the original liquid content after centrifugation.  $\text{LHC}_2$  is the amount of liquid related to the dry matter content. The inner and outer circles represent 50 % and 100 % explained variance, respectively. PC 1 (component 1) explains 32 % of  $X$  and 6 % of  $Y$ , while PC 2 (component 2) explains 20 % of  $X$  and 7 % of  $Y$  (validated results). Adapted from paper III.

The first component (factor 1) mainly explained the difference between the four groups. In particular, a difference between small and large fish can be seen and this may be related to differences in e.g. lipid contents. The second component (factor 2) was related to a development during chill storage of the smoked samples and the greatest change was seen

---

between day 11 and day 20. This change was related to a change in the liquid holding capacity as a decrease in the liquid holding capacity was observed between day 11 and day 20 after smoking ( $p < 0.01$ ) (Figure 3.6). This suggests that the difference observed in water distribution during cold storage of smoked salmon is related to important quality changes (Paper III).

A significant difference between the group of small fish and large fish regarding water pool III was found for both intact and minced samples (Paper I). This result indicates that water pool III is affected by the size of the fish, though the actual effect of changes in water pool III in relation to quality aspects is unknown. A relationship between water pool III, lipid content and large fish from farm 1 was observed in Figure 3.6. The size of water pool III did not change during chill storage.

To conclude, three water pools were identified for minced and intact salmon with almost equal sets of relaxation times. The sizes of water pools I and II were affected by processing and chill storage of the smoked product. Changes in the water distribution during chill storage were concurrent with changes in the liquid holding capacity. The water distribution also reflected the use of different salting and smoking parameters as differences between in-house smoked and commercially smoked products were seen. Thus, the measurement of water distribution is a promising tool for optimising the product quality of smoked salmon, although the marked differences between the two intact samples clearly illustrates the heterogeneity of the fillet and the importance of an accurate and well-considered sampling.

### **3.10 Changes in microstructure**

Changes in microstructure due to raw material, smoking process and chill storage of the smoked product were studied by light microscopy (LM) and confocal laser scanning microscopy (CLSM). Only the salmon samples from experiment 2 were examined.

Several methods were tested in order to find a suitable microscopy technique to study structural changes and to investigate the lipid distribution in raw and smoked salmon. Before the actual analysis, the main focus was to find a suitable staining method for staining lipids when using a light microscope. An oil red staining method was tried. However problems regarding spreading of lipid (droplets) on the surface of the specimen

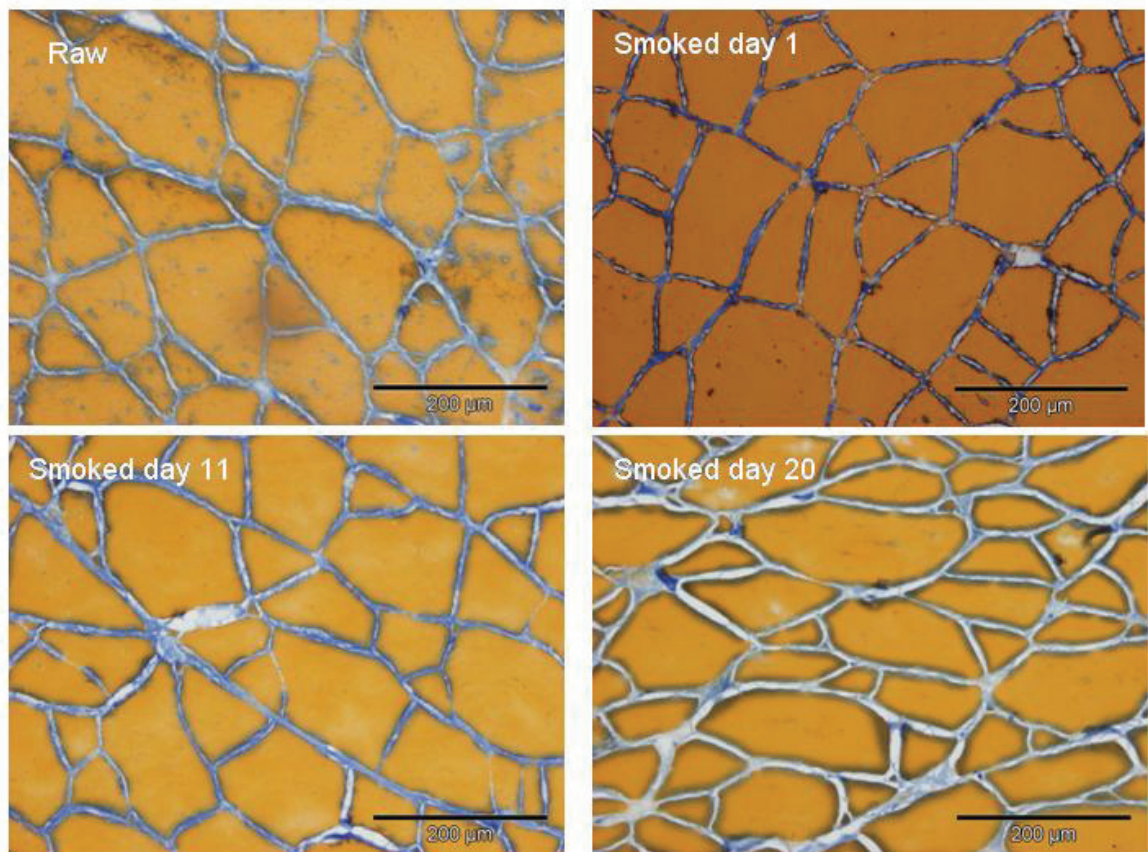


---

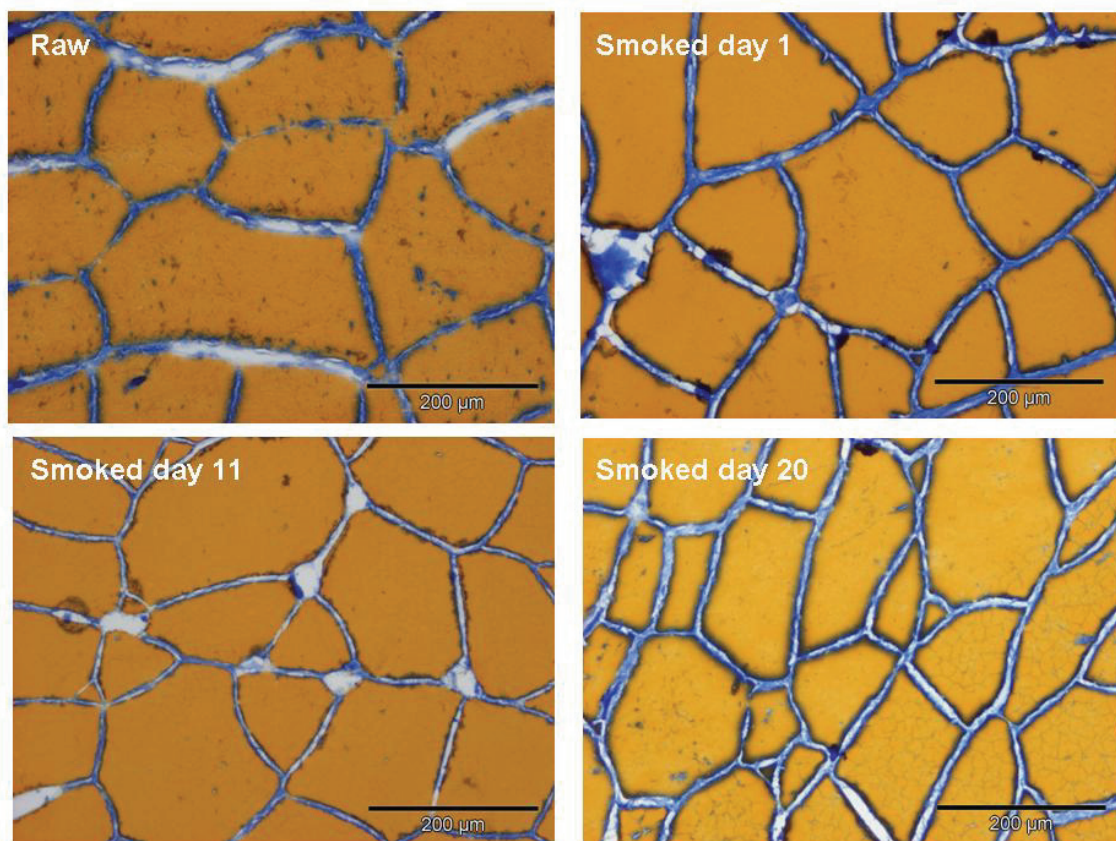
were observed. The spreading of lipids on the surface could take place during the cutting of the small cubes or during the cutting at the freezing microtome. Therefore, other microscopy techniques e.g. fluorescence microscopy and confocal laser scanning microscopy (CLSM) were tried to investigate the lipid distribution. The CLSM appear to be the most promising method. The CLSM does not depend on the transmitting of light through the specimen, but uses a focused scanning laser to illuminate a sub-surface layer of the specimen. Therefore, problems regarding the spreading of lipid on the surface are not the same. In addition, CLSM does not require as thin sections as the LM. Different staining methods were tested and a Nile blue staining method was found to be a suitable method to stain lipid. Only preliminary CLSM studies using visual comparisons of the images were carried out.

Images from LM of cross-sections of whole muscle from a small salmon (lipid content 11.5 %) and from a large salmon (lipid content 17.1 %), raw, day 1, day 11 and day 20 after smoking are shown in Figure 3.7 and Figure 3.8, respectively. Muscle cells are stained yellow and collagen and gelatine are stained blue. To get an indication of differences between the raw and smoked samples in a simple and easy way, the number of cells along a diagonal line, drawn across the image, was counted. The counting of the cells showed that the raw samples had fewer cells (in the range of 5-6) compared to the smoked samples (in the range of 8-10). Thus, the muscle cells seem to be more tightly bound after the smoking process. Both the salting step and the subsequent smoking step that includes heating at 20°C to 30°C can affect the properties of muscle proteins and thereby the muscle structure. Sigurgisladóttir *et al.* (2000a), Sigurgisladóttir *et al.* (2000b) and Sigurgisladóttir *et al.* (2001) found that muscle fibres shrank during the salting and smoking process. This was measured by calculating the number of muscle cells as a percentage of the total number of cells in a defined cross-sectional area.

Changes in microstructure during the chill storage period were seen as the extracellular space between the cells became wider after 20 days of chill storage of the smoked product (Figure 3.7 and 3.8). However the shape of the cells was still intact and there was no shrinkage of cells even after 20 days of storage.

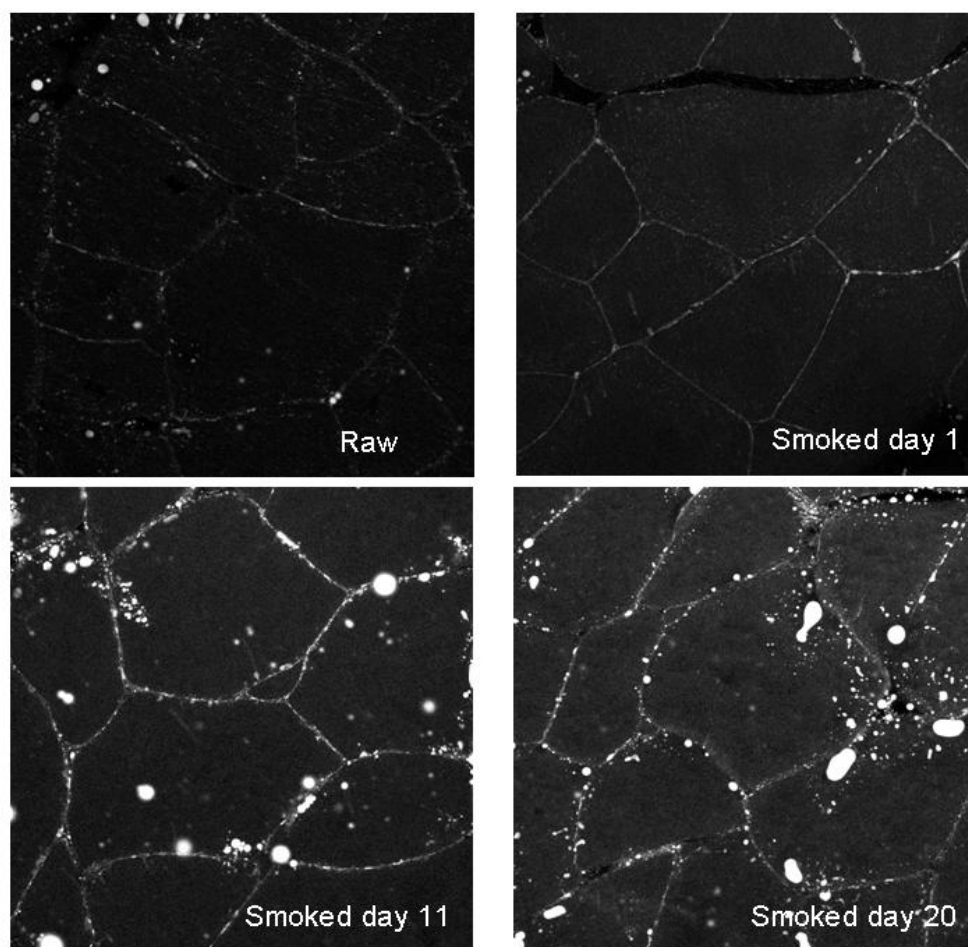


**Figure 3.7.** Images obtained by LM of cross-sectioned whole muscle from a small salmon: raw, day 1, day 11 and day 20 after smoking. Sections are stained for collagen (blue) and muscle protein (yellow). Adapted from Paper III.



**Figure 3.8.** Images obtained by LM of cross-sectioned whole muscle from a large salmon: raw, day 1, day 11 and day 20 after smoking. Sections are stained for collagen (blue) and muscle protein (yellow). Adapted from Paper III.

Images from confocal laser scanning microscopy (CLSM) (Figure 3.9) of the large salmon samples (stained for lipids with Nile blue) indicated the same pattern in the changes during chill storage of the smoked salmon as observed with the LM images (Figure 3.8). The CLSM images were made in the Meat Science Department at the Faculty of Life Science, University of Copenhagen (unpublished results).



**Figure 3.9.** Images obtained by confocal laser scanning microscope of cross-sectioned whole muscle from a large salmon: raw, day 1, day 11 and day 20 after smoking. Sections are stained for lipids with Nile blue.

Sigurgisladóttir *et al.* (2001) found that lipids in smoked salmon muscle were released from the fat cells and floated freely as fat droplets between the muscle cells. Looking at the LM images (Figure 3.7 and 3.8), there appear to be larger spots in the extracellular space after 20 days of chill storage, which could be an indication of more released lipid from the cells into the extracellular space. For the CLSM images (Figure 3.9), it appears that more fat droplets between the muscle cells were visible after 11 days of chill storage.

The denaturation and shrinkage temperatures of collagen are near 20 and 40°C, respectively (Sikorski *et al.* 1984). Liquid loss between 20 and 35°C could be due to the denaturation of collagen. This alters the physical properties of the pericellular layer, which represents a physical barrier to the release of fluid (Ofstad, 1995). In the present study, a decreased liquid holding capacity was observed during chill storage of smoked salmon. It

---

might be possible that changes in collagen during the cold smoking process affect the liquid holding capacity in the muscle due to an alteration of the physical properties of the pericellular layer (Ofstad *et al.* 1995; Elvevoll *et al.* 1996).

In conclusion, the microstructure was affected by the whole process. The muscle cells became smaller and the extracellular space between the cells became wider during chill storage. Indications of released lipid droplets in the extracellular space were found.

---

## Chapter 4

### 4. Conclusion and perspectives

The importance of the lipid content, raw material and storage time on quality parameters like the liquid holding capacity in raw and smoked product was investigated. Also the suitability of different methods to determine the liquid holding capacity was examined. The liquid holding capacity is a complex property which is influenced by several factors and more specifically it is concluded that

- The size of the fish affected the liquid holding capacity in smoked fish as large fish lost more liquid than smaller fish. It was also found that the lipid content increased with the size of the fish. The salt content influenced the liquid holding capacity as a high salt content gave a higher liquid holding capacity. The salt uptake of the fillets was affected by the lipid content as a high lipid content lead to lower salt content. The lipid content affected the liquid holding capacity in raw salmon, as a high lipid content gave lower liquid holding capacity. Thus, the lipid content is an important parameter regarding the liquid holding capacity as it can influence the liquid holding capacity directly or in-directly by affecting other factors e.g. the salt content which influences the liquid holding capacity.
- During chill storage of the smoked product the liquid holding capacity decreased and this was mainly seen between 11 and 20 days of chill storage. At the same time the lipid fraction of the liquid loss increased while the water fraction of the liquid loss remained constant. During chill storage, changes in the water pool sizes were observed, which indicates that the water pool size was related to a decrease in the liquid holding capacity. During chill storage indications of released lipid droplets in the extracellular space were found, which could indicate denaturation of the collagen structure. Thus, the higher liquid loss during storage could be due to weakening of the structure/denaturation of the collagen.
- This study showed that the centrifugation method and the liquid leakage test had different applicability as the two methods provide supplementary rather than redundant information. Thus, it is recommended that both types of methods are used in order to get as detailed a picture as is possible of this important quality parameter. The liquid holding capacity determined by the centrifugation method was predicted successfully from the NMR relaxation curves for both salmon and rainbow trout.

---

## **Perspectives**

The production of farmed salmon has grown substantially during the recent years and the lipid level in farmed fish has increased. At the same time, problems regarding liquid loss, discolouration and soft texture have been reported. In this study, it has been shown that it is possible to produce smoked salmon products without severe liquid loss in a pilot scale production using a controlled raw material. During the experiments carried out in this project, no excessive liquid loss was observed even after 20 days of chill storage of the smoked product. This could indicate that the production of smoked salmon using a controlled raw material can give a cold smoked salmon of high quality regarding liquid loss. It is therefore of outmost importance that the smoke houses use standards regarding variation in raw material especially for the lipid content.

Many other parameters than the ones included in this thesis might be important for the liquid loss of cold smoked salmon. Future work should also focus on storage time and temperature of the raw material before production and on freezing and its effect on the cold smoked product. Freezing can be included in several ways as the smoke houses may choose to freeze their fish either before or after processing. In some production methods, the smoked fillet is also short-term frozen to facilitate the slicing of the smoked fillet.

---

## 5. References

- Aidos, I., Lie, Ø. and Espe, M. (1999). Collagen content in farmed Atlantic salmon (*Salmo Salar* L.). Journal of Agricultural and Food Chemistry, 47: 1440-1444.
- Aknes, A., Gjerde, B. and Roald, S. O. (1986). Biological, chemical and organoleptic changes during maturation of salmon (*Salmo salar*). Aquaculture, 53: 7-20.
- Andersen, C. A. and Jørgensen, B. M. (2004). On the relation between water pools and water holding capacity in cod muscle. Journal of Aquatic Food Product Technology, 13 (1): 13-23.
- Ando, M., Toyohara, H., Shimizu, Y. and Sakaguchi, M. (1991). Post-mortem tenderization of fish muscle proceeds independently of resolution of rigor mortis. Bulletin of the Japanese Society of Scientific Fisheries [Nihon Suisan Gakkai-shi], 57 (6): 1165-1169.
- Ando, M., Toyohara, H. and Sakaguchi, M. (1992). Three-dimensional structure of collagen fibrillar network of pericellular connective tissue in association with firmness of fish muscle. Bulletin of the Japanese Society of Scientific Fisheries [Nihon Suisan Gakkai-shi], 58 (7): 1361-1364.
- AOAC. (2000). Method no. 976.18 "Salt (Chlorine as Sodium Chloride) in Seafood. Potentiometric Method". Official Methods of Analysis, AOAC, Arlington, VA.
- AOAC. (2000). Method no. 937.07 "Fish and marine products. Treatment and preparation of sample procedure". Official Methods of Analysis, AOAC, Arlington, VA.
- AOAC. (2000). Method no. 971.27 "Sodium chloride in canned vegetables. Potentiometric method". Official Methods of Analysis, AOAC, Arlington, VA.
- AOCS Official method Ce 2-66. (1998a). Preparation of methyl esters of long-chain fatty acids. Champaign IL: AOCS.
- AOCS Official method Ce 1b-89. (1998b). Fatty acid composition by GLC. Marine Oils. Champaign IL: AOCS.
- Aursand, M., Blevik, B., Rainuzzo, J. R., Jørgensen, L. and Mohr, V. (1994). Lipid distribution and composition of commercially farmed Atlantic salmon (*Salmo salar*). Journal of the Science of Food and Agriculture, 64: 239-248.
- Auty, M. A. E., Twomey, M., Guinee, T. P. and Mulvihill, D. M. (2001). Development and application of confocal scanning laser microscopy methods for studying the distribution of fat and protein in selected dairy products. Journal of Dairy Research, 68: 417-427.
- Bell, J. G., McEvoy, J., Webster, J. L., McGhee, F., Millar, R. M. and Sargent, J. R. (1998). Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo salar*). Journal of Agricultural and Food Chemistry, 46: 119-127.
- Bell, J. G., McEvoy, J., Tocher, D. R., McGhee, F., Campbell, P. J. and Sargent, J. R. (2001). Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. Journal of Nutrition, 131: 535-543.
- Bell, J. G., Henderson, J., Tocher, D. R., McGhee, F., Dick, J. R. Porter, A., Smullen, R. P. and Sargent, J. R. (2002). Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle



---

fatty acid composition and hepatic fatty acid metabolism. *Journal of Nutrition*, 132: 220-230.

Birkeland, S. (2004). Cold-smoking of Atlantic salmon (*Salmo salar*) – Influence of processing conditions and raw material characteristics on quality and yield. Doctor Thesis, Department of Animal and Aquacultural Sciences, Agricultural University of Norway.

Birkeland, S. and Bjerkeng, B. (2005). The quality of cold-smoked Atlantic salmon (*Salmo salar*) as affected by salting method, time and temperature. *International Journal of Food Science and Technology*, 40: 963-976.

Birkeland S., Rørå, A. M. B., Skåra T. and Bjerkeng, B. (2003). Product yield and gaping in cold-smoked Atlantic salmon (*Salmo salar*) fillets as influenced by different injection-salting techniques. *Journal of Food Science*, 68(5): 1743-1748.

Birkeland, S., Røra, A. M. B., Skåra, T. and Bjerkeng, B. (2004a). Effects of cold smoking procedures and raw material characteristics on product yield and quality parameters of cold smoked Atlantic salmon (*Salmo salar* L.) fillets. *Food Research International*, 37: 273-286.

Birkeland, S., Haarstad, I., and Bjerkeng, B. (2004b). Effects of salt-curing procedure and smoking temperature on astaxanthin stability in smoked salmon. *Journal of Food Science*, 69: 198-203.

Bjerkeng, B. (2000). Carotenoid pigmentation of salmonid fishes – recent progresss. In: *Avances en Nutrición Acuicola V. Memorias del V Simposium Internacional de Nutrición Acuicola* (Eds.: Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M. A. and Civera-Cerecedo, R.). 19-22. November, Mérida, Yucatán. pp 71-89.

Bjerkeng, B. (2004). Colour deficiencies in cold-smoked Atlantic salmon. *I dybden*, 23 (2): 401-413.

Bjerkeng, B., Storebakken, T. and Liaaen-Jensen, S. (1992). Pigmentation of rainbow trout from start feeding to sexual maturation. *Aquaculture*, 108: 333-346.

Bjerkeng, B., Refstie, S., Fjalestad, K. T., Storebakken, T., Rødbotten, M. and Roem, A. J. (1997). Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. *Aquaculture*, 157: 297-309.

Bligh, E. G. and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37: 911-917.

Blonk, J. C. G. and van Aalst, H. (1993). Confocal scanning light microscopy in food research. *Food Research International*, 26: 297-311.

Bremner, H. A. (1985). A convenient easy-to-use system for estimating the quality of the chilled seafood. In: *Proceedings of the fish processing conference* (Eds.: Scott, D. N. and Summers, G.). Nelson, New Zealand 23-25 April. *Fish Processing Bulletin*, 7: 59-63.

Bremner, H. A. (1992). Fish flesh structure and the role of collagen - its post-mortem aspects and implications for fish processing. In: *Quality assurance in the fish industry* (Eds.: Huss, H. H., Jakobsen, M., Liston, J.). Amsterdam, London, New York, Tokyo, Elsevier. pp 41-62.

Bremner, H. A. (1999). Gaping in fish flesh. In: *Extracellular matrix of fish and shellfish* (Eds.: Sato, K., Sakaguchi, M. and Bremner, H. A.). Research signpost, Trivandrum, India, Chapter VII, pp 81-93.

Bremner, H. A. (2000). Toward practical definitions of quality for food science. *Critical Reviews in Food Science and Nutrition*, 40 (1): 83-90.

- 
- Bremner, H. A. and Hallett, I. C. (1985). Muscle fiber- connective tissue junctions in the fish blue grenadier (*Macrurus novaezelandiae*). A scanning electron microscope study. *Journal of Food Science*, 50: 975-980.
- Bro, R. (1997). PARAFAC. Tutorial and applications. *Chemometrics and Intelligent Laboratory Systems*, 38: 149-171.
- Brooker, B. E. (1991). The study of food systems using confocal laser scanning microscopy. *Microscopy and Analysis*, 12: 13-15.
- Brooker, B. E. (1995). Imaging food systems by confocal laser scanning microscopy. In: *New physico-chemical techniques for the characterization of complex food systems* (Ed.: Dickinson, E.), Blackie Academic and Professional. London. pp 53-68.
- Cardinal, M., Knockaert, C., Torrissen, O. J., Sigurgisladottir, S., Mørkøre, T., Thomassen, M. S. and Vallet, J. L. (2001). Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Research International*, 34: 537-550.
- Cardinal, M., Gunnlaugsdottir, H., Bjørnevik, M., Ouisse, A., Vallet, J. L. and Leroi, F. (2004). Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. *Food Research International*, 37: 181-193.
- Carr, H. Y. and Purcell, E. M. (1954). Effect of diffusion free precession in nuclear magnetic resonance experiments. *Physical Review*, 94: 630-638.
- Carrol, J. D. and Chang, J. (1970). Analysis of individual differences in multidimensional scaling via an N-way generalization of "Eckart-Young" decomposition. *Psychometrika*, 35: 283-319.
- Cogswell, C. J. and Sheppard, C. J. R. (1990). Confocal brightfield imaging techniques using an on-axis scanning optical microscope. In: *Confocal Microscopy* (Ed.: Wilson, T.). Academic Press, London. pp 213-243.
- Colquhoun, I. J. (1993). NMR spectroscopy in food science. *Nutrition and Food Science*, 1: 8-12.
- Corzo, O. and Bracho, N. (2004). Effects of brine concentration and temperature on equilibrium distribution coefficients during osmotic dehydration of sardine sheets. *Lebensmittel-Wissenschaft und- Technologie*, 37: 475-479.
- Deleanu, C. and Paré, J. R. J. (1997). Nuclear magnetic resonance spectroscopy (NMR): Principles and applications. In: *Instrumental methods in food analysis* (Eds.: Paré, J. R. J. and Belanger, J. M. R.). Elsevier Science, Amsterdam. pp 179-236.
- DFU (2005). Forsynings- og markedsundersøgelse af akvakulturprodukter. Internal report.
- Diaz, G., Wolf, W., Kostaropoulos, A. E., and Spiess, W. E. L. (1993). Diffusion of low-molecular compounds in food model systems. *Journal of Food Processing and Preservation*, 17: 437-454.
- Doe, P., Sikorski, Z., Haard, N. F., Olley, J. and Pan, B. S. (1998). Basic principles: In: *Fish drying and smoking. Production and quality* (Ed.: Doe, P.). Lancaster, Pennsylvania, USA: Technomic Publishing Company Inc. pp 13-45.
- Dunajski, E. (1979). Texture of fish muscle. *Journal of Texture Studies*, 10: 301-318.
- Eckhoff, K. M., Aidos I., Hemre, G-I. and Lie, Ø. (1998). Collagen content in farmed Atlantic salmon (*Salmo salar* L.) and subsequent changes in solubility during storage on ice. *Food Chemistry*, 62 (2): 197-

---

200.

Eckhoff, K. M., Aidos, I., Hemre, G-I. and Lie, Ø. (1998). Collagen content in farmed Atlantic salmon (*Salmo salar*, L.) and subsequent changes in solubility during storage on ice. *Food Chemistry*, 62(2): 197-200

Eide, O., Børresen, T. and Strøm, O. (1982). Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from fatty species. *Journal of Food Science*, 47: 347-349, 354.

Einen, O., Mørkøre, T., Rørå, A. M. B. and Thomassen, M. S. (1999). Feed ration prior to slaughter - a potential tool for managing product quality of Atlantic salmon (*Salmo salar*). *Aquaculture*, 178 (1-2): 149-169.

Elvevoll, E. O., Sørensen, N. K., Østerud, B., Ofstad, R. and Martinez, I. (1996). Processing of marine foods. *Meat Science*, 43: 265-275.

Engelsen, S. B. and Bro, R. (2003). PowerSlicing. *Journal of Magnetic Resonance*, 163 (1): 192-197.

Erikson, U., Sigholt, T. and Seland, A. (1997). Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*), *Aquaculture*, 149: 243-252.

Esbensen, K. H. (2000). Multivariate data analysis – in practice. An introduction to multivariate data analysis and experimental design. CAMO ASA, Oslo, Norway.

Espe, M., Nortvedt, R., Lie, Ø. and Hafsteinsson, H. (2002). Atlantic salmon (*Salmo salar* L.) as raw material for the smoking industry. II: Effects of different smoking methods on losses of nutrients and on the oxidation of lipids. *Food Chemistry*, 77 (1): 41-46.

Espe, M., Ruohonen, K., Bjørnevik, M., Frøyland, L., Nortvedt, R. and Kiessling, A. (2004). Interactions between ice storage time, collagen composition, gaping and textural properties in farmed salmon muscle harvested at different times of the year. *Aquaculture*, 240: 489-504.

FAO/WHO (1983). Codex Alimentarius. Recommended international code of practice for smoked fish. Codex alimentarius. Vol B CAC/RCP 25-1979, 1-35. Food and Agriculture Organization of the United Nations World Health Organization.

Farrar, T. C. (1989). Pulsed NMR spectroscopy. The Farragut press, Madison.

Field, L. D. (1989). Fundamental aspects of NMR spectroscopy. In: *Analytical NMR*, (Eds.: Field, L. D. and Sternhell, S.). John Wiley and sons, Chichester. pp 5-39.

Fjelkner-Modig, S. and Tornberg, E. (1986). Water distribution in porcine (*M. longissimus dorsi*). *Meat Science*, 17: 213-231.

Francis, F. J. (1995). Quality as influenced by color, *Food Quality and preferences*, 6: 149-155.

Gallert-Jornet, L., Barat, J. M., Rustad, T., Erikson, U., Escrìche, I. and Fito, P. (2007). A comparative study of brine salting of Atlantic cod (*Gadus morhus*) and Atlantic salmon (*Salmo salar*). *Journal of Food Engineering*, 79: 261-270.

Gjedrem, T. (1997). Flesh quality improvement in fish through breeding. *Aquaculture International*, 5: 197-206.

- 
- Gómez-Guillén, M. C., Montero, P., Hurtado, O. and Borderías, A. J. (2000). Biological characteristics affect the quality of farmed Atlantic salmon and smoked muscle. *Journal of Food Science*, 65: 53-60.
- Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S. J., Sandberg, M. G., Standal, H. and Røsjø, C. (2002). Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. *Aquaculture*, 207: 311-329.
- Hallett, I. C. and Bremner, H. A. (1988). Fine structure of the myocommata-muscle fibre junction in hoki (*Macruronus novaezelandiae*). *Journal of the Science of Food and Agriculture*, 44: 245-261.
- Hamm, R. (1986). Functional properties of the myofibrillar system and their measurements. In: *Muscle as food* (Ed. Bechtel, P. J.). Academic Press Inc, New York, pp 135-199.
- Hansen, L. T. (1995). Quality of chilled, vacuum packed cold-smoked salmon. Ph. D. thesis. Royal Veterinary and Agricultural University of Denmark.
- Hansen, L. T., Gill, T. and Huss, H. H. (1995). Effects of salt and storage temperature on chemical, microbiological and sensory changes in cold-smoked salmon. *Food Research International*, 28 (2): 123-130.
- Hansen, L. T., Gill, T., Rontved, S. D. and Huss, H. H. (1996). Importance of autolysis and microbiological activity on quality of cold-smoked salmon. *Food Research International*, 29 (2): 181-188.
- Hardy, R. W. and King, I. B. (1989). Variation in n-3 fatty acid content of fresh and frozen salmon. *Omega 3 News*, 4: 1-4.
- Hardy, R. W., Scott, T. M. and Harrell, L. W. (1987). Replacement of herring oil with menhaden oil, soybean oil or tallow in the diets of Atlantic salmon raised in marine net-pens. *Aquaculture*, 65: 267-277.
- Harshman, R. A. (1970). Foundations of the PARAFAC procedure; Model and conditions for an “exploratory” multimode factor analysis. *UCLA Working Papers in Phonetics*, 16: 1-84.
- Harshman, R. A. and Lundy, M. E. (1994). PARAFAC: Parallel factor analysis. *Computational Statistics and Data Analysis*, 18: 39-72.
- Hatae, K., Tobimatsu, A., Takeyama, M. and Matsumoto, J. J. (1986). Contribution of the connective tissue on the texture difference of various fish species. *Bulletin of the Japanese Society of Scientific Fisheries*, 52 (11): 2001-2007.
- Hemminga, M. A. (1992). Introduction to NMR. *Trends in Food Science and Technology*, 3: 179-186.
- Hemre, G. I. and Sandnes, K. (1999). Effect of dietary lipid level on muscle composition in Atlantic salmon (*Salmo salar*). *Aquaculture Nutrition*, 5: 9-16.
- Hermansson, A. M. (1986). Water- and fat holding. In: *Functional properties of food macromolecules* (Eds.: Mitchell, J. R. and Ledward, D. A.). London: Elsevier Ltd. pp 273-314.
- Hills, B. (1998). *Magnetic Resonance Imaging in Food Science*. Wiley & Sons, New York.
- Horner, W. F. A. (1992). Preservation of fish by curing (drying, salting and smoking). In: *Fish processing technology* (ed. Hall, G. M.). Blackie Academic and Professional, Glasgow UK. pp 31-71.
- Howgate, P. (1979). Fish. In: *Food Microscopy* (ed.: Vaughan, J.). Academic Press, London, pp 343-389.

- 
- Hyldig, G. and Nielsen, J. (2004), QIM - a tool for determination of fish freshness, In: *Seafood Quality and Safety. Advances in the New Millennium* (Eds.: Shahidi, F. and Simpson, B. K.), ScienceTech Publishing Company, St John, NL. pp. 81-89.
- Hyldig, G. and Green-Petersen, D. M. B. (2005). Quality index Method – an objective tool for determination of sensory quality. *Journal of Aquatic Food Product Technology*, 13 (4): 71-80.
- Højmarklaboratoriet. (2005). Information omkring metoder til måling af vædetab i lakseråvare og koldrøget laks. Højmarklaboratoriet a/s - Rapport nr. 60.
- ISO 3496, SA. (1994). Meat and Meat products – determination of L (-) Hydroxyproline content, 2<sup>nd</sup> edition. International Organisation for Standardisation, Geneva.
- Jepsen, S. M., Pedersen, H. T. and Engelsen, S. B. (1999). Application of chemometrics to low-field relaxation data of intact fish flesh. *Journal of the Science of Food and Agriculture*, 73: 1793-1802.
- Jensen, K. N. and Jørgensen, B. M. (2003). Effect of storage conditions on differential scanning calorimetry profiles from thawed cod muscle. *Lebensmittel-Wissenschaft und- Technologie*, 36: 807-881.
- Jensen, K. N., Guldager, H. S. and Jørgensen, B. M. (2002). Three way modelling of NMR relaxation profiles from thawed cod muscle. *Journal of Aquatic Food Product Technology*, 11 (3/4): 201-214.
- Jensen, K. N., Jørgensen, B. M., Nielsen, J. and Nielsen, H. H. (2005). Water distribution and mobility in herring muscle in relation to lipid content, season, fishing ground and biological parameters. *Journal of the Science of Food and Agriculture*, 85: 1259-1267.
- Jittinandana, S., Kenney, P. B., Slider, S. D. and Kiser, R. A. (2002). Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. *Journal of Food Science*, 67 (6): 2095-2099.
- Jónsdóttir, S. (1992). Quality index method and TQM system. In: *Quality issues in the fish industry. Proceedings from the COMETT conference course: Quality issues in the fish industry, european cooperation* (Eds.: Olafsson, R. and Ingthorsson, A. H.). Reykjavik Iceland, September. pp 81-94.
- Katikou, P., Hughes, S. I. and Robb, D. H. F. (2001). Lipid distribution within Atlantic salmon (*Salmo Salar*) fillets. *Aquaculture*, 202: 89-99.
- Koteng, D. F. (1992). Markedsundersøkelse, Norsk laks. Published by Fiskerinæringsens Landsforening (FNL), Norway.
- Kris-Etherton, P. M., Harris, W. S. and Appel, L. J. (2002). Fish consumption, fish oil, omega-3 fatty acids and cardiovascular diseases. *American Heart Association Circulation*, 106: 2747-2757.
- Lavéty, J., Afolobi, O. A. and Love, R. M. (1988). The connective tissue of fish. IX. Gaping in farmed species. *International Journal of Food Science and Technology*, 23: 23-30.
- Lie, Ø., Sandvik, A. and Waagø, R. (1993). Influence of dietary fatty acids on the lipid composition of lipoproteins in farmed Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry*, 12: 249-260.
- Love, R. M. (1970). The chemical biology of fishes. New York: Academic Press.
- Love, R. M. (1988). The food fishes: Their intrinsic variation and practical implication. Farrand Press, London.
- Mackie, I. M., (1993). The effect of freezing on flesh proteins. *Food Reviews International*, 9: 575-610.

- 
- Maga, J. A. (1987). Smoke in food processing. CRC Press Inc. Boca Raton, FL.
- Mannan, A., Fraser, D. I. and Dyer, W. J. (1961). Proximate composition of Canadian Atlantic fish I. Variation in composition of different sections of the flesh of Atlantic halibut (*Hippoglossus hippoglossus*). Journal of Fish Research BD. Canada, 18: 483-493.
- Martens, H. and Martens, M. (2001). Multivariate analysis of quality. An introduction. Jon Wiley & Sons, Chichester.
- Martens, H. and Næs, T. (1989). Multivariate calibration. John Wiley and sons, England.
- Martinsdóttir, E., Sveinsdóttir, K., Luten, J., Schelvis-Smit, R. and Hyldig, G. (2001). Reference manual for the fish sector: Sensory evaluation of fish freshness. QIM-Eurofish, The Netherlands.
- Meiboom, S. and Gill, D. (1958). Modified spin-echo method for measuring nuclear times. The Review of Scientific Instruments, 29: 688-691.
- Meiselman, H. (2001). Criteria of food quality in different contexts. Food Service Technology, 1: 67-84.
- Michie, I. (2001). Causes of downgrading in the Salmon farming industry. In: *Farmed fish quality* (Eds.: Kerstin S. C. and Warriss, P. D.). Bristol, UK: Fishing News Books, Blackwell Science Ltd. pp 129-136.
- Morrissey, P. A., Mulvihill, D. M. and O'Neill, E. M. (1987). Functional properties of muscle proteins. In: *Developments in Food proteins – 5* (Ed.: Hudson, B. J. F). Elsevier, London, pp 195-256.
- Mørkøre, T. and Rørvik, K. A. (2001). Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (*Salmo salar*) transferred to seawater as 0 + smolts or 1+ smolts Aquaculture, 199: 145-157.
- Mørkøre, T., Vallet, J. L., Cardinal, M., Gómez-Guillén, M. C., Montero, P., Torrissen, O. J., Nordtvedt, R., Sigurgisladottir, S. and Thomassen, M. S. (2001). Fat content and fillet shape of Atlantic salmon: Relevance for processing yield and quality of raw and smoked products. Journal of Food Science, 66 (9): 1348-1354.
- Mørkøre, T., Hansen, A. Å., Unander, E. and Einen, O. (2002). Composition, liquid leakage and mechanical properties of farmed rainbow trout: Variation between fillets sections and the impact of ice and frozen storage. Journal of Food Science, 67: 1933-1938.
- Nilsson, K. and Ekstrand, B. (1994). Refreezing rate after glazing affects cod and rainbow trout muscle tissue. Journal of Food Science, 59: 797-799.
- Offer, G. and Trinick, J. (1983). On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Science, 8: 245-281.
- Offer, G., Knight, O., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp, A., Starr, R. and Purslow, P. (1989). The structural basis of water-holding, appearance and toughness of meat and meat products. Food Microstructure, 8: 151-170.
- Ofstad, R. (1995). Microstructure and liquid-holding capacity in cod (*Gadus morhua* L.) and salmon (*Salmo salar*) muscle; Effects of heating. Dr. Scient. Thesis. Institute of Medical Biology, University of Tromsø.
- Ofstad, R., Kidman, S., Myklebust, R. and Hermansson, A. M. (1993). Liquid holding capacity and structural changes during heating of fish muscle; cod (*Gadus morhua* L.) and salmon (*Salmo salar*) Food.Structure, 12: 163-174.

- 
- Ofstad, R., Kidman, S., Myklebust, R., Olsen, R. L. and Hermansson, A. M. (1995). Liquid holding capacity and structural changes in comminuted salmon (*Salmo salar*) muscle as influenced by pH, salt and temperature. *Lebensmittel-Wissenschaft und- Technologie*, 28: 329-339.
- Ofstad, R., Kidman, S., Myklebust, R., Olsen, R. L. and Hermansson, A. M. (1996). Factors influencing liquid-holding capacity and structural changes during heating of comminuted cod (*Gadus morhua* L.) muscle. *Lebensmittel-Wissenschaft und- Technologie*, 29: 173-183.
- Olsen, S. H., Sorensen, N. K., Stonno, S. K. and Elvevoll, E. O. (2006). Effect of slaughter methods on blood spotting and residual blood in fillets of Atlantic salmon (*Salmo salar*). *Aquaculture*, 258: 462-469.
- Pedersen, H. T. (2001). Low-field nuclear magnetic resonance and chemometrics applied in food science. Ph. D. thesis. Royal Veterinary and Agricultural University of Denmark.
- Pedersen, H. T., Bro, R. and Engelsen, S. B. (2001). SLICING – a novel approach for unique deconvolution of NMR relaxation decays. In: *Magnetic resonance in food science: A view to the future* (Eds.: Webb, G. A., Belton Toward, P. S., Gill, A. M., Degadillo, I.). The Royal Society of Chemistry, Cambridge. pp 202-209.
- Pedersen, H. T., Bro, R. and Engelsen, S. B. (2002). Towards Rapid and Unique Curve Resolution of Low-Field NMR relaxation data: Trilinear SLICING versus Two-dimensional Curve Fitting. *Journal of Magnetic Resonance*, 157: 141-155.
- Rasmussen, R. S. (2001). Quality of farmed salmonids with emphasis on proximate composition, yield and sensory characteristics. *Aquaculture Research*, 32: 767-786.
- Refsgaard, H. F. F., Brockhoff, P. and Jensen, B. (1998). Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (*Salmo salar*). *Journal of Agricultural and Food Chemistry*, 46: 808-812.
- Regenstein, J. M., Jauregui, C. A. and Baker, R. C. (1984). The effect of pH, polyphosphates and different salts on water retention properties of ground trout muscle. *Journal of Food Biochemistry*, 8: 123-131.
- Regost, C., Arzel, J., Cardinal, M., Laroche, M. and Kaushik, S. J. (2001). Fat deposition and flesh quality in seawater reared triploid brown trout (*Salmo trutta*) as affected by dietary fat levels and starvation. *Aquaculture*, 193: 325-345.
- Regost, C., Jakobsen, J. V. and Rørå, A. M. B. (2004). Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. *Food Research International*, 37: 259-271.
- Robb, D. H. F., Kestin, S. C., Warriss, P. D. and Nute, G. R. (2002). Muscle lipid content determines the eating quality of smoked and cooked Atlantic salmon (*Salmo salar*). *Aquaculture*, 205: 345-358.
- Ruan, R. R. and Chen, P. L. (1998). *Water in Foods and Biological Materials. A Nuclear Magnetic Approach*. Technomic Publishing Company, Lancaster.
- Rørå, A. M. B. (2003). Raw material characteristics and treatment – effects on yield and quality of cold-smoked Atlantic salmon. Ph. D. thesis. Agricultural University of Norway.
- Rørå, A. M. B. and Einen, O. (2003). Effects of freezing on quality of cold-smoked salmon based on the measurements of physiochemical characteristics. *Journal of Food Science*, 68 (6): 2123-2128.
- Rørå, A. M. B. and Regost, C. (2003). Liquid holding capacity of farmed Atlantic salmon muscle at low temperature heating - effect of frozen and ice storage. *Journal of Aquatic Food Product Technology*, 12(3):

- Rørå, A. M. B., Kvåle, A., Mørkøre, T., Rørvik, K.-A., Steinen, S. H. and Thomassen, M. S. (1998). Process yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*) in relation to raw material characteristics. *Food Research International*, 31 (8): 601-609.
- Rørå, A. M. B., Regost, C. and Lampe, J. (2003). Liquid holding capacity, texture and fatty acid profile of smoked fillets of Atlantic salmon fed diets containing fish oil or soybean oil. *Food Research International*, 36 (3): 231-239.
- Rørå, A. M. B., Furuhaug, R., Fjæra, S. O. and Skjervold, P. O. (2004). Salt diffusion in pre-rigor filleted Atlantic salmon. *Aquaculture*, 232: 255-263.
- Rørå, A. M. B., Ruyter, B., Skorve, J., Berge, R. K. and Slinning, K-E. (2005a). Influence of high content of dietary soybean oil on quality of large fresh, smoked and frozen Atlantic salmon (*Salmo salar*). *Aquaculture International*, 13: 217-231.
- Rørå, A. M. B., Birkeland, S., Hultmann, L., Rustad, T., Skåra, T. and Bjerkeng, B. (2005b). Quality Characteristics of farmed Atlantic salmon fed diets high in soybean or fish oil as affected by cold smoking temperature. *Journal of Food Science*, 38: 201-211.
- Sargent, J. R., Bell, J. G., McGhee, F., McEvoy, J. and Webster, J. L. (2001). The nutritional value of fish. In: *Farmed fish quality* (Eds.: Kestin, S. C. and Warris, P.). Blackwell, Oxford. pp 3-12.
- Sato, K., Yoshinaka, R., Sato, M. and Shimizu, T. (1986). Collagen content in muscle of fishes in association with their swimming movement and meat texture. *Bulletin of the Japanese Society of Scientific Fisheries*, 52 (9): 1595-1600.
- Sato, K., Ohashi, C., Ohtsuki, K. and Kawabata, M. (1991). Type V collagen in trout (*Salmo gairdneri*) muscle and its solubility change during chilled storage of muscle. *Journal of Agricultural and Food Chemistry*, 39: 1222-1225.
- Schnepf, M. (1989). Protein-water interactions. In: *Water and food quality* (Ed.: Hardman, T. M.). Elsevier Applied Science, London. pp 135-168.
- Shearer, K. D., Åsgård, T., Andorsdottir, G. and Aas, G. H. (1994). Whole body elemental and proximate composition of Atlantic salmon (*Salmo salar*) during the life cycle. *Journal of Fish Biology*, 44: 785-797.
- Sheehan, E. M., O'Connor, T. P. O., Sheehy, P. J. A., Buckley, D. J. and FitzGerald, R. (1996). Effect of dietary fat intake on the quality of raw and smoked salmon. *Irish Journal of Agricultural and Food Research*, 35 (1): 37-42.
- Shenderyuk, V. I. and Bykowski, P. J. (1990). Salting and marinating of fish. In: *Seafood: resources, nutritional composition and preservation* (Ed.: Sikorski, Z. E.). CRC Press Inc. Boca Raton, Florida, pp 147-162.
- Shomer, I., Weinberg, Z. G. and Vasiliver, R. (1987). Structural binding properties of silvercarp (*Hypophthalmichthys molitrix*) muscle affected by NaCl and CaCl<sub>2</sub> treatments. *Food Microstructure*, 6: 199-207.
- Sigurgisladóttir, S., Torrissen, Lie, Ø. Thomasson, M. and Hafsteinsson, H. (1997). Salmon quality: Methods to determine the quality parameters. *Reviews in Fisheries Science*, 5: 223-252.
- Sigurgisladóttir, S., Hafsteinsson, H., Jonsson, A., Lie, Ø., Nordvedt, R., Thomasson, M. and Torrissen, O.



---

1999. Textural properties of raw salmon fillets as related to sampling method. *Journal of Food Science*, 64(1): 99-104

Sigurgisladóttir, S., Ingvarsdóttir, H., Torrissen, O. J., Cardinal, M. and Hafsteinsson, H. (2000a). Effect of freezing/thawing on the microstructure and the texture of smoked Atlantic salmon (*Salmo salar*). *Food Research International*, 33 (10): 857-865.

Sigurgisladóttir, S., Sigurdardóttir, M. S., Torrissen, O. J., Vallet, J. L. and Hafsteinsson, H. (2000b). Effects of different salting and smoking processes on the microstructure, the texture and yield of Atlantic salmon (*Salmo salar*) fillets. *Food Research International*, 33: 847-855.

Sigurgisladóttir, S., Sigurdardóttir, M. S., Ingvarsdóttir, H., Torrissen, O. J., and Hafsteinsson, H. (2001). Microstructure and texture of fresh and smoked Atlantic salmon, *Salmo salar* L., fillets from fish reared and slaughtered under different conditions. *Aquaculture Research*, 32: 1-10.

Sikorski, Z. E., Scott, D. N. and Buisson, D. H. (1984). The role of collagen in the quality and processing of fish. *CRC Critical Reviews in Food Science and Nutrition*, 20: 301-343.

Sikorski, Z. E., Kolakowska, S. and Pan, B. S. (1990). The nutritive composition of the major groups of marine food organism. In: *CRC seafood: resources, nutritional composition and preservation* (Ed. Sikorski Z. E.), CRC Press, Boca Raton, Florida, pp 29-54.

Skrede, G. and Storebakken, T. (1986a). Characteristics of color in raw, baked and smoked wild and pen-reared Atlantic salmon. *Journal of Food Science*, 51 (3): 804-808.

Skrede, G. and Storebakken, T. (1986b). Instrumental color analysis of raw, baked and smoked wild and pen-reared Atlantic salmon. *Aquaculture*, 53 (3-4): 279-286.

Storebakken, T., Hung, S. S. O., Calvert, C. C. and Plisetskaya, E. M. (1991). Nutrient partitioning in rainbow trout at different feeding rates. *Aquaculture*, 96: 191-203.

Sveinsdóttir, K., Martinsdóttir, E., Hyldig, G., Jørgensen, B. and Kristbergsson, K. (2002). Application of Quality Index Method (QIM) scheme in shelf life study of farmed Atlantic salmon (*Salmo salar*). *Journal of Food Science*, 67 (4): 1570-1579.

Sveinsdóttir, K., Hyldig, G., Martinsdóttir, E., Jørgensen, B. and Kristbergsson, K. (2003). Development of Quality Index Method (QIM) scheme for farmed Atlantic salmon (*Salmo salar*). *Food Quality and Preferences*, 14: 237-245.

The Danish Directorate of Fisheries (2005). *Yearbook of Fishery Statistics 2005*.

Torrissen, O. J. (1995). Hvordan har pigmenteringen av norsk laks utviklet sig? *Norsk Fiskeopdrett*, 18A: 12-13.

Torrissen, O. J., Sigurgisladóttir, S. and Slinde, E. (2001). Texture and technological properties of fish. In: *Farmed Fish Quality* (Eds.: Kestin, S. C and Warris, P. ). Blackwell science, Oxford. pp 42-57.

Torstensen, B. E., Bell, J. G., Rosenlund, G., Henderson, J., Graff, I. E., Douglas, R., Tocher, D., Lie, Ø. and Sargent, J. R. (2005). Tailoring of Atlantic Salmon (*Salmo salar* L.) Flesh Lipid Composition and Sensory Quality by Replacing Fish Oil with a Vegetable Oil Blend. *Journal of Agricultural and Food Chemistry*, 53 (26): 10166 - 10178.

Wang D., Tang, J., Correia L. R. and Gill T. A. (1998). Post-mortem changes in cultivated Atlantic salmon

---

and their effects on salt uptake. *Journal of Food Science*, 63 (4): 634-637.

Wang D., Tang, J. and Correia, L. R. (2000). Salt diffusivities and salt diffusion in farmed Atlantic salmon muscle as influenced by rigor mortis. *Journal of Food Engineering*, 43, 115-123.

Windig, W. and Antaluk, B. (1997). Direct exponential curve resolution algorithm (DECRA): A novel application of the generalized rank annihilation method for a single spectral mixture data set with exponentially decaying contribution profiles. *Chemometrics and Intelligent Laboratory Systems*, 37: 241-254.

Wold, S., Esbensen, K. and Geladi, P. (1987). Principal component analysis. *Chemometrics and Intelligent Laboratory Systems*, 2: 37-52.

# Water distribution in smoked salmon

Hanne Løje\*, Ditte Green-Petersen, Jette Nielsen, Bo M Jørgensen and Kristina N Jensen

Danish Institute for Fisheries Research, Department of Seafood Research, DTU, Building 221, DK-2800 Kgs. Lyngby, Denmark

**Abstract:** Low-field  $^1\text{H}$  nuclear magnetic resonance (NMR) tranverse relaxations were measured on intact and minced muscle from raw and smoked salmon differing in size (small or large) and storage days after smoking (1, 11 and 20 days). Water distribution in the samples was calculated from the NMR signals by the use of multi-way data analysis. For intact and minced salmon samples, three water ‘pools’ with  $T_2$  values of 38, 50 and 182 ms and of 33, 50 and 183 ms, respectively, were identified. For both intact and minced samples, the size of water pool I and II were affected by storage time after smoking, whereas the size of pool III was affected by the size of the fish. Intact fillet samples taken near the skin and near the middle of the fillet differed with respect to shape of the NMR relaxation signal reflecting a non-uniform distribution of the size of the three water pools in intact fillet.

© 2006 Society of Chemical Industry

**Keywords:** fish; NMR relaxation; PARAFAC; storage time; raw material

## INTRODUCTION

Traditionally, cold smoked salmon is a highly estimated product. The cold smoking process includes salting and heating and has not changed fundamentally over a decade. There has, however, been an increasing tendency towards a lower water holding capacity in recent years.<sup>1</sup> A lower water holding capacity results in a higher liquid loss. A high liquid loss is economically disadvantageous to the producer and can lead to a decreased consumer acceptance of cold smoked salmon. It is therefore important to know how the main constituents in the fish can vary due to the properties of the raw material and the processing steps in order to obtain the required product quality.

Water is the main constituent of muscle and many important quality parameters such as water holding capacity, storage stability and texture are related to the interaction of water with protein and the distribution of water in the muscle tissue.<sup>2–5</sup> The muscle water can be considered as distributed in several domains or ‘pools’ according to its interaction with and/or entrapment by structural elements of the cells.<sup>4</sup> The distribution of water between these pools depends on changes in chemical composition and physical structure<sup>4,5</sup> as well as on storage and processing parameters, such as storage time, freezing and mincing. Knowledge of water distribution in raw and cold smoked salmon muscle, therefore, provides important information about how changes in the raw material properties are related to quality differences.

Low-field  $^1\text{H}$  nuclear magnetic resonance (NMR) is an excellent tool for measuring the water distribution in food systems as it is a rapid and non-destructive technique.  $^1\text{H}$  NMR measures properties of protons

and is therefore a direct technique for investigating the total quantity of water and the state of water and its interactions in the fish muscle tissue. NMR has been used in several studies to determine functional properties of food systems. In fish, low-field NMR has been used to study the effect of different process parameters on cod muscle,<sup>6–9</sup> and to determine the oil and water contents in herring<sup>10</sup> and salmon.<sup>11,12</sup> NMR has also been used for the determination of water holding capacity in cod<sup>12,13</sup> and for mapping the raw material properties of herring in relation to location and time of catch.<sup>14</sup>

The NMR tranverse relaxation signal in heterogeneous material is composed of a sum of mono-exponentials, indicating that several populations of water molecules with different magnetic relaxation properties exist. The successful application of multi-way chemometrics to low-field NMR relaxation of food samples has shown the unique decomposition of the signal into underlying mono-exponential relaxation curves, each of which represents a water pool.<sup>6,13–16</sup>

In the present study, the multi-way technique was used to determine the number and the size of water pools from NMR relaxation profiles in raw and cold smoked salmon. Changes in water distribution were related to raw material properties, such as fish size, and processing parameters, i.e. chill storage and smoking.

## MATERIALS AND METHODS

### Experimental design

In the experiment both raw (unprocessed) and smoked salmon were analysed. The smoked samples were

\* Correspondence to: Hanne Løje, Danish Institute for Fisheries Research, Department of Seafood Research, Technical University of Denmark, Building 221, DK-2800, Kgs. Lyngby, Denmark  
E-mail: hal@difres.dk

Contract/grant sponsor: The Danish Ministry of Food, Agriculture and Fisheries

(Received 1 September 2005; revised version received 21 December 2005; accepted 18 August 2006)

Published online 2 November 2006; DOI: 10.1002/jsfa.2693

© 2006 Society of Chemical Industry. J Sci Food Agric 0022–5142/2006/\$30.00

stored chilled for up to 20 days after smoking. Samples were taken for analysis three times during the storage period.

The fish used were Atlantic salmon (*Salmo salar*) reared in Norway and harvested in May 2004. After slaughtering the fish were transported in ice to the Danish Institute for Fisheries Research (DIFRES) in Denmark. The fish arrived at DIFRES on day 6 after slaughter, the day processing was started.

The fish came from two farms. From each farm, there were two sizes of fish. One group consisted of small salmon with a size of 3–4 kg and the other group of large salmon with a size of 6–7 kg. Thus, there was a total of four groups (Table 1).

### Preparation of samples

The fish were filleted by hand and trimmed. The left fillet was kept as raw (unprocessed) and cut into four pieces (Fig. 1). Piece one (in front of the dorsal fin) was used for NMR measurements (intact and minced samples).

The right fillet was dry-salted (60 g of salt per kg fillet) for 5 h for small fish (size 3–4 kg) and 8 h for the large fish (size 6–7 kg) at room temperature. The fillets were rinsed and afterwards chilled at 2 °C until the next day. The fillets were dried for 2 h and smoked in an

oven supplied with smoke generated from beechwood, for 5½ h at 26 °C. After smoking the fillets were chilled at 2 °C until the next day.

The smoked fillet side was cut into the same four pieces as its raw counterpart (Fig. 1). For the smoked fish, piece one (in front of the dorsal fin) was further divided into three smaller pieces. Each of the three smaller pieces was vacuum-packed and randomly assigned to one of three storage periods (1, 11 or 20 days) at 2 °C after smoking. On each day of analysis, NMR measurements were performed on intact and minced samples. These samples are referred to as in-house smoked samples.

### Commercial samples

Three fillets from salmon of size 3–4 kg from farm two were dry-salted and smoked in a traditional oven at a commercial smokehouse in Denmark. Both the salting and smoking process were longer than for the in-house smoked samples. Afterwards the fillets were sent to DIFRES. The samples were cut into the same pieces as the in-house smoked salmon (Fig. 1). Analysis was performed on day 6 and day 15 after smoking. The samples are referred to as commercial samples.

### Nuclear magnetic resonance measurements

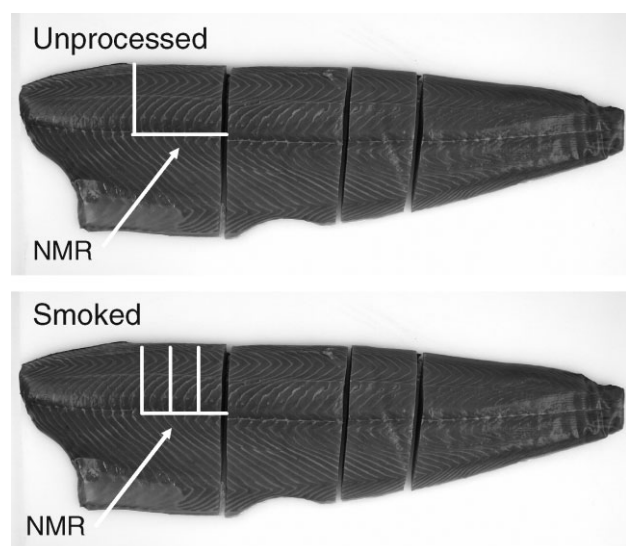
The NMR measurements were performed on samples from the part in front of the dorsal fin. Only the loin part was used. The origin of the NMR samples is shown in Fig. 2. First two muscle cubes (named intact samples), each of approximately 2 g were cut and weighed into small cylindrical glass tubes that fitted into 18 mm NMR sample tubes. The first muscle cube (called sample A) was cut about 2 mm from the skin, avoiding the dark muscle (Fig. 2). The second muscle cube (called sample B) was cut nearer to the skin than sample A. The rest of the piece was minced for 2 × 5 s at 5 °C in a Knifetec, 1095 Sample Mill (Foss Tecator, Sweden). Portions of 2 g mince were weighed into a small cylindrical glass tube that matched the inner diameter of the 18 mm NMR sample tube.

Low-field NMR relaxation measurements were performed on a Maran Benchtop Pulsed NMR analyser (Resonance Instruments, Witney, UK), operating at 23.2 MHz and equipped with an 18 mm variable temperature probe head. Transverse

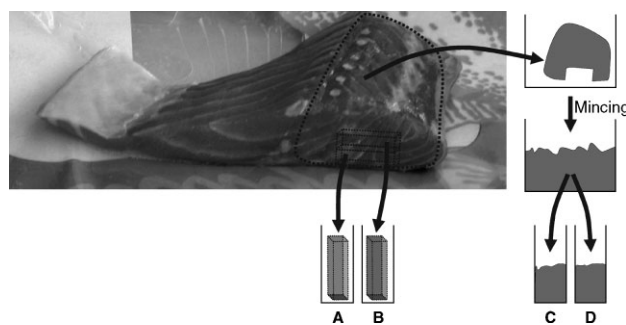
**Table 1.** Overview of salmon samples

Code	Description	Number of fish	Analysis day
1	Farm 1, small	7	Raw <sup>a</sup> , 1, 11 and 20 days after smoking
2	Farm 2, small	6	Raw <sup>a</sup> , 1, 11 and 20 days after smoking
3	Farm 1, large	2	Raw <sup>a</sup> , 1, 11 and 20 days after smoking
4	Farm 2, large	3	Raw <sup>a</sup> , 1, 11 and 20 days after smoking

<sup>a</sup> The raw fish were analysed 7 days after slaughtering.



**Figure 1.** Sampling of unprocessed and smoked salmon.



**Figure 2.** Sampling of intact samples and minced samples for NMR measurements.

relaxation was measured using the CPMG pulse sequence.<sup>17,18</sup> The relaxation data were acquired as eight scan repetitions using a 6 s relaxation delay. The inter-pulse spacing  $\tau$  in the CPMG sequence was 200  $\mu$ s and the number of data points acquired was 512, spaced by  $4\tau$  (even echoes). The sample probe temperature was kept constant at 8 °C by a continuous flow of dry air and the samples were thermo equilibrated for at least 30 min at 8 °C before measurements.

### Data analysis

The NMR relaxation curves were normalised to equal sample weight and the number of points reduced by a factor of 4 (taking means of neighbour points), resulting in 128 data points per sample. A preliminary principal component analysis (PCA) revealed one outlying measurement on the intact salmon samples and three on the minced salmon samples. These measurements were removed before further analysis. The data analysis also revealed that the first five sample points in the reduced NMR relaxation profile were excessively noisy (resulting in disturbed loadings, probably due to an instrumental artefact).<sup>6</sup> These five data points were therefore removed leaving 123 data points. The PCA and partial least squares (PLS) regressions were calculated by column mean-centred data and full cross validation using The Unscrambler<sup>®</sup> version 9.1 (CAMO, Oslo, Norway). Statistical tests (*t*-test) were made using GraphPad Prism version 4 (GraphPad Software, San Diego, CA, USA).

The data matrix containing NMR replicates was converted to a three-directional array made up of 7 'slabs'. The first slab containing columns 1–91; slab 2, columns 2–92; slab 3, columns 3–93; slab 4, columns 5–95; slab 5, columns 9–99; slab 6, columns 17–107; and slab 7, columns 33–123. More information on tri-linearization NMR relaxation data can be found in Pedersen *et al.*<sup>19</sup> and Engelsen and Bro.<sup>20</sup> Parallel factor analysis (PARAFAC) was calculated on the resulting array as described in Jensen *et al.*<sup>6</sup> using Matlab<sup>®</sup> version 7.04 (Math Works, Natick, MA, USA) and freeware Matlab<sup>®</sup> code available at <http://www.models.kvl.dk>.

The number of PARAFAC components was determined by splitting the data set into two between replicates (for the minced samples) and by random selection into two data sets (for the intact samples). The loadings were inspected as these should be mono-exponential and equal in the two sets. The number of components was determined as the maximum number giving equal mono-exponential loadings in the two subsets.

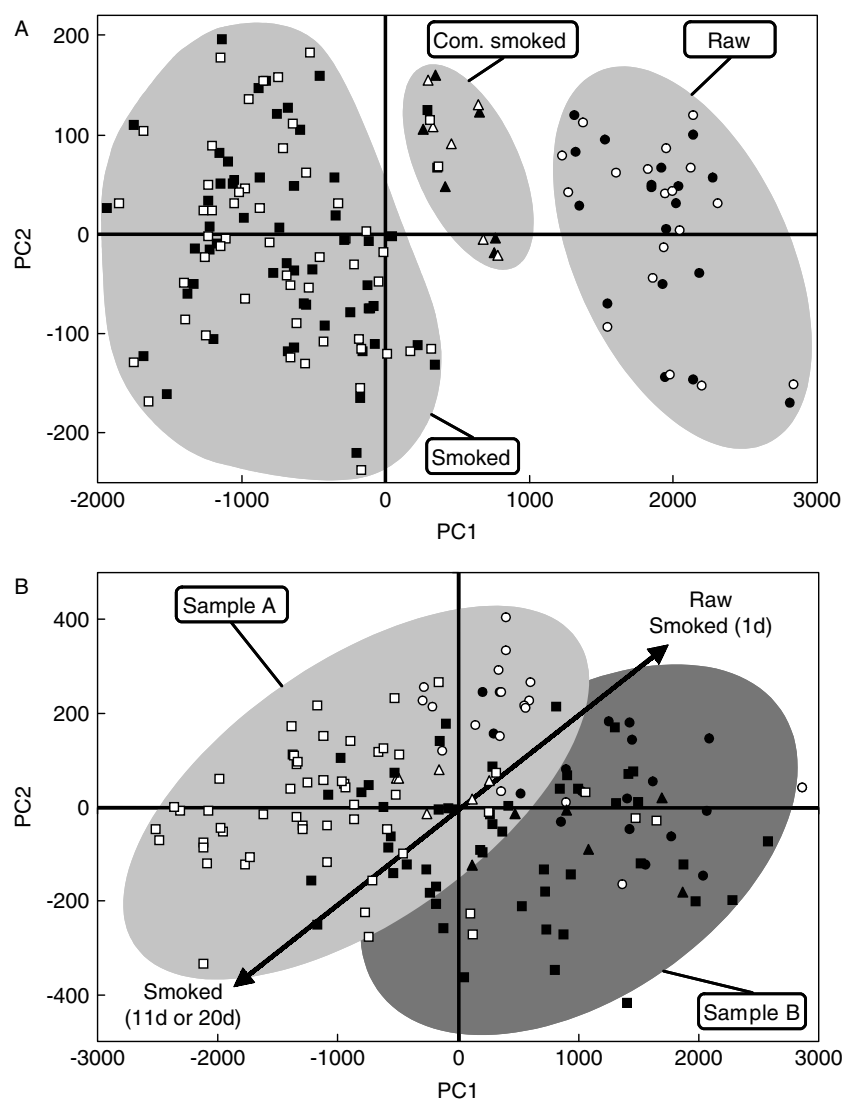
## RESULTS AND DISCUSSION

### Sampling

In order to explore the overall variation in the NMR relaxation profiles of salmon, PCA models

were calculated on minced and intact samples. In Fig. 3, scores plots from the minced and intact salmon samples are shown. For the minced samples, the first principal component differentiated between raw and smoked salmon (Fig. 3A) indicating that the salting and smoking process, not surprisingly, affected the water distribution. Furthermore, a third group of commercial smoked samples placed between the raw and the smoked could be identified (Fig. 3A). These commercial smoked salmon are from the same batch as the small fish from farm two, but the cold-smoking process (salting and smoking) was different, which apparently has affected the water distribution. Another interesting point is that two of the in-house smoked samples have scores lying within the group of commercial samples. When inspecting the third PCA component (not shown), a grouping of the smoked salmon samples according to initial storage time (1 day) and longer storage time (11 and 20 days) was revealed. In order to reveal whether the difference in NMR signal between raw and smoked salmon was purely due to variation in water content, the relaxation profiles were normalised to maximum amplitude. This did not change the overall patterns for minced and intact samples, however, indicating that the main variations represented in the scores plot (Fig. 3) were not related to differences in water content but due to differences in water distribution.

For the intact samples (Fig. 3B), the pattern was somewhat different than for the minced samples as the unprocessed and the commercial samples did not form separated groups. Instead, a group consisting of unprocessed samples, initially smoked samples (1 day of storage) and commercial samples separated from the smoked salmon stored for longer time (11 or 20 days). Another aspect was observed, as the samples tended to separate according to sample A and sample B. The reason for this is probably due to the heterogeneity of the salmon fillets. The samples were always cut side by side, but sample A was cut approximately 2 mm from the skin (avoiding dark muscle in the sample), whereas sample B was always cut nearest to the middle of the fillet (Fig. 2). This sampling may lead to different chemical compositions of the two samples as the lipid content is usually higher (and the water content lower) near the skin.<sup>21</sup> Thus, an explanation to the sample pattern, i.e. different water distribution between sample A and B, seen in Fig. 3B, could be a difference in chemical composition with sample A having a higher lipid content than sample B. Refsgaard *et al.*<sup>22</sup> showed that the distribution of lipid varied throughout the salmon fillet, which illustrated a lack of uniformity of the fillet. In cod, the sampling position within the fillet was also shown to affect the water distribution,<sup>15</sup> even though the lipid content in cod is very low and not expected to differ significantly throughout the fillet. The present results clearly show the effects of sampling, and that even small distances between two close sampling points can result in marked differences.



**Figure 3.** Component 2 versus component 1 scores from a PCA on NMR relaxation profiles of salmon. (A) Raw and smoked minced samples from salmon. Raw replicate C (●) and D (○); commercially smoked replicate C (▲) and D (△); in-house smoked replicate C (■) and D (□). (B) Raw and smoked intact muscle samples from salmon. Same symbols used as in (A) with filled symbols representing sample A and empty symbols representing sample B. The points are also systematically distributed according to storage time as indicated by the arrows.

### Water distribution

The first and most important step when decomposing the NMR relaxation profiles with PARAFAC is to determine the number of components, which, in this case, corresponds to the number of water pools. The samples were divided into two data sets consisting of minced and intact samples respectively. The commercial samples were included in both sets.

Calculating PARAFAC models on the two data sets revealed that both minced samples and intact samples contained three water pools. The estimated  $T_2$  values are given in Table 2. These results were supported by split-half analysis, i.e. splitting each data set in two and recalculating PARAFAC models for each subset.

In the study by Jepsen *et al.*,<sup>12</sup>  $T_2$  values of 39, 84 and 353 ms were obtained for fresh, minced salmon after exponential fitting. In the present study,  $T_2$  values of 33, 50 and 183 ms were found for minced (raw and smoked) salmon by using PARAFAC modelling.

**Table 2.** Relaxation times of water pools in minced and intact samples from raw and smoked salmon

Sample set	Pool I $T_2$ (ms)	Pool II $T_2$ (ms)	Pool III $T_2$ (ms)
Intact muscle	38	50	182
Minced	33	50	183

The differences between the results of the two studies may be due to variations in the sample preparation, lipid content and the method of data analysis. In addition the present study also included smoked salmon.

Other studies of  $T_2$  values in fish muscles have been published recently. Nott *et al.*<sup>23</sup> used magnetic resonance imaging (MRI) to determine  $T_2$  in fresh (45 and 121 ms) and frozen/thawed trout (44 and 129 ms). Jensen *et al.*<sup>6,14</sup> used low-field NMR and identified four water pools (37, 56, 126 and 361 ms) in samples from frozen/thawed minced cod and two water

pools (37 and 75 ms) in samples from frozen/thawed summer herring and three water pools (41, 58 and 102 ms) in samples from frozen/thawed winter herring. Steen and Lambelet<sup>8</sup> reported three water pools (1, 39 and 135 ms) in minced cod. Jepsen *et al.*<sup>12</sup> reported  $T_2$  values of 37, 79 and 448 ms in frozen/thawed cod. Comparing the relaxation times reported in the literature for fish muscle samples show a fair agreement except for the very fast relaxing component reported by Steen and Lambelet.<sup>8</sup>

After having determined the number of water pools and their  $T_2$  values, the size of each water pool was calculated. As the absolute relaxation amplitudes are proportional to the amount of sample (or water) present, the relative amplitudes within samples were used. The relative water distribution in the minced salmon samples shown in Table 3 revealed that the size of water pool II was significantly higher and water pool I was significantly lower in raw samples compared with in-house smoked samples ( $P < 0.0001$ ). The commercial samples had also significantly higher content of water in pool II and significantly lower content of water pool I compared to the in-house smoked sample ( $P < 0.0001$ ), but they were also significantly different from the raw samples. These results indicate that water distribution is affected by the cold smoking process. For the in-house smoked samples, a decrease in the size of water pool II and a parallel increase in the size of water pool I was seen during the chill storage from day 1 to day 20 after smoking with the greatest change happening between 1 day and 11 days of storage after smoking. A similar change in water distribution was also observed during chill storage of thawed cod packed in modified atmosphere, where increase in size of water pool I at the expense of pool II was found to correspond to the denaturation of muscle protein and a simultaneously change in pH.<sup>24</sup> In this study, the change in size of

water pool I and II corresponded to an increase in liquid loss from 1 day of storage to 20 days of storage (unpublished results). This suggests that the observed difference in water distribution during cold storage of smoked salmon is related to important quality changes.

In commercial samples, the sizes of water pool I and II were between the values for raw samples and initially in-house smoked samples (1 day) even though the commercial samples had been stored for up to 15 days. The commercial samples had been through a different salting and smoking process, which had included longer salting at lower temperature and longer smoking time. The smoking was carried out in a traditional oven, where the temperature was slowly raised. This procedure may be more gentle resulting in less denaturation of proteins compared to the in-house smoked salmon, where the differences between the products are being reflected in the water distribution.

In the intact samples, the three water pools identified were identical for A and B samples, while the water distribution were different between A and B samples. The relative water distribution for small and large fish for intact A and B samples are shown in Table 4. The intact A samples had higher content of water in pool II, while the intact B samples had higher content of water in pool I ( $P < 0.0001$ ). A significant difference between the group of small fish and large fish according to water pool III were found for both intact and minced samples. This result indicate that water pool III is affected by the size of the fish, though the actual effect of changes in water pool III in relation to quality aspects is unknown.

## CONCLUSION

The results have shown that salting and smoking influenced the water distribution in salmon. Three water pools were determined for minced and intact salmon with almost equal sets of relaxation times. The sizes of water pools I and II were affected by processing and chill storage of the smoked product, and may be related to important quality changes like liquid loss. The water distribution also reflected the use of different salting and smoking parameters as differences between in-house smoked and commercial smoked products were seen. Thus, the measurement of water distribution is a promising tool for optimising product quality of smoked salmon, although the

**Table 3.** Relative water pool sizes in minced salmon samples

	Number of samples	Pool I		Pool II		Pool III	
		Mean	SD	Mean	SD	Mean	SD
Raw	32	0.12	0.07	0.82	0.07	0.06	0.01
Day 1	36	0.51	0.09	0.44	0.09	0.05	0.01
Day 11	36	0.60	0.08	0.36	0.08	0.05	0.01
Day 20	35	0.64	0.09	0.31	0.09	0.06	0.01
Commercial	12	0.33	0.05	0.62	0.05	0.05	0.01

**Table 4.** Relative water pool sizes in intact salmon samples

Sample <sup>a</sup>	Size	Number of samples	Pool I		Pool II		Pool III	
			Mean	SD	Mean	SD	Mean	SD
Intact A	Small	54	0.40	0.21	0.55	0.21	0.05	0.01
	Large	20	0.41	0.23	0.52	0.23	0.07	0.01
Intact B	Small	45	0.60	0.21	0.36	0.20	0.04	0.01
	Large	19	0.59	0.20	0.36	0.20	0.06	0.01

<sup>a</sup> Labels A and B refer to Fig. 2.

marked differences between the two intact samples clearly illustrated the heterogeneity of the fish fillet and the importance of an accurate and well-considered sampling.

## ACKNOWLEDGEMENTS

This work was financially supported by grants from The Danish Ministry of Food, Agriculture and Fisheries. The authors are very thankful to Mr Carsten Østerberg for assistance with salting and smoking, and to Mr Ulrik Cold for technical assistance with NMR measurements.

## REFERENCES

- 1 Rørå AMB and Einen O, Effects of freezing on quality of cold-smoked salmon based on the measurements of physicochemical characteristics. *J Food Sci* **68**:2123–2128 (2003).
- 2 Offer G, Knight O, Jeacocke R, Almond R, Cousins T, Elsey J, *et al*, The structural basis of water-holding, appearance and toughness of meat and meat products. *Food Microstruct* **8**:151–170 (1989).
- 3 Fjelkner-Modig S and Tornberg E, Water distribution in porcine *M. longissimus dorsi* in relation to sensory properties. *Meat Sci* **17**:213–231 (1986).
- 4 Hills B, *Magnetic Resonance Imaging in Food Science*. Wiley & Sons, New York (1998).
- 5 Ruan RR and Chen PL, *Water in Foods and Biological Materials. A Nuclear Magnetic Approach*. Technomic Publishing Company, Lancaster (1998).
- 6 Jensen KN, Guldager HS and Jørgensen BM, Three way modelling of NMR relaxation profiles from thawed cod muscle. *J Aquat Food Prod Technol* **11**(3/4):201–214 (2002).
- 7 Lambelet P, Renevey F, Kaabi C and Raemy A, Low-field nuclear magnetic resonance relaxation study of stored or processed cod. *J Agric Food Chem* **43**:1462–1466 (1995).
- 8 Steen C and Lambelet P, Texture changes in frozen cod mince measured by low-field nuclear magnetic resonance spectroscopy. *J Sci Food Agric* **75**:268–272 (1997).
- 9 Erikson U, Veliyulin E, Singstad TE and Aursand M, Salting and desalting of fresh and frozen-thawed cod (*Gadus morhus*) fillets: A comparative study using  $^{23}\text{Na}$  NMR,  $^{23}\text{Na}$  MRI, low-field  $^1\text{H}$  NMR and physicochemical analytical methods. *J Food Sci* **69**:107–114 (2004).
- 10 Nielsen D, Hyldig G, Nielsen HH and Nielsen J, Lipid content in herring (*Clupea harengus* L.) – Influence of biological factors and comparison of different methods of analyses: solvent extraction, Fatmeter, NIR and NMR. *Food Sci Technol* **38**:537–548 (2005).
- 11 Bechmann IE, Pedersen HT, Nørgaard L and Engelsen SB, Comparative chemometric analysis of transverse low-field  $^1\text{H}$  NMR relaxation data, in *Advances in Magnetic Resonance in Food Science*, ed. by Belton PS, Hills BP and Webb GA. The Royal Society of Chemistry, Cambridge, pp. 217–225 (1999).
- 12 Jepsen SM, Pedersen HT and Engelsen SB, Application of chemometrics to low-field  $^1\text{H}$  NMR relaxation data of intact fish flesh. *J Sci Food Agric* **73**:1793–1802 (1999).
- 13 Andersen CA and Jørgensen BM, On the relation between water pools and water holding capacity in cod muscle. *J Aquat Food Prod Technol* **13**(1):13–23 (2004).
- 14 Jensen KN, Jørgensen BM, Nielsen J and Nielsen HH, Water distribution and mobility in herring muscle in relation to lipid content, season, fishing ground and biological parameters. *J Sci Food Agric* **85**:1259–1267 (2005).
- 15 Andersen CA and Rinnan Å, Distribution of water in fresh cod. *Lebens-Wiss Technol* **35**:687–696 (2002).
- 16 Povlsen VT, Rinnan Å, van der Berg F, Andersen HJ and Thybo A, Direct decomposition of NMR relaxation profiles and prediction of sensory attributes of potato samples. *Lebens-Wiss Technol* **36**:423–432 (2003).
- 17 Carr HY and Purcell EM, Effect of diffusion free precession in nuclear magnetic resonance experiments. *Am J Physiol* **94**:630–638 (1954).
- 18 Meiboom S and Gill D, Modified spin-echo method for measuring nuclear times. *Rev Sci Instr* **29**:688–691 (1958).
- 19 Pedersen HT, Bro R and Engelsen SB, Towards rapid and unique curve resolution of low-field NMR relaxation data: Trilinear SLICING versus two-dimensional curve fitting. *J Magn Res* **157**:141–155 (2002).
- 20 Engelsen SB and Bro R, Powerslicing. *J Magn Res* **163**:192–197 (2003).
- 21 Mannan A, Fraser DI and Dyer WJ, Proximate composition of Canadian Atlantic fish I. Variation in composition of different sections of the flesh of Atlantic halibut (*Hippoglossus hippoglossus*). *J Fish Res Bd Canada* **18**:483–493 (1961).
- 22 Refsgaard HFF, Brockhoff P and Jensen B, Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (*Salmo salar*). *J Agric Food Chem* **46**:808–812 (1998).
- 23 Nott KP, Evans SD and Hall LD, Quantitative magnetic resonance imaging of fresh and frozen-thawed trout. *Magn Reson Imag* **17**:445–455 (1999).
- 24 Jensen KN and Jørgensen BM, Effect of storage conditions on differential scanning calorimetry profiles from thawed cod muscle. *Food Sci Technol* **36**:807–881 (2003).



1    **Quality of cold smoked salmon**

2        -    **Reviewing the influence of variations in raw material and**

3            **technological parameters**

4

# **Quality of cold smoked salmon**

## **- Reviewing the influence of variations in raw material and technological parameters**

Hanne Løje and Jette Nielsen

Hanne Løje, MSc, and Jette Nielsen MSc, are affiliated with Technical University of Denmark, Danish Institute for Fisheries Research, Department of Seafood Research, Søtofts Plads, Building 221, DK-2800 Kgs. Lyngby, Denmark

Address correspondence to: Hanne Løje, Technical University of Denmark, Danish Institute for Fisheries Research, Department of Seafood Research, Søtofts Plads, Building 221, DK-2800 Kgs. Lyngby, Denmark (e-mail: [hal@difres.dk](mailto:hal@difres.dk))

The study was financially supported by the Danish Ministry of Food, Agriculture and Fisheries.

### **Abstract**

The salmon smoking industry has recently been facing new problems such as high frequency of gaping, soft texture, low and uneven colour distribution and liquid leakage in the smoked product. As a result, the image of cold smoked salmon is declining, leading to considerable financial loss for the producers. The biggest change in production has been the replacement of wild salmon by farmed salmon. Farmed salmon

1 differ from wild salmon by having a higher lipid content due to the feeding regime.  
2 High variations in lipid content have been reported and, since the lipid content can  
3 affect several parameters such as yield, texture, colour and liquid holding capacity,  
4 information on the lipid content is important for the industry in order to ensure a  
5 consistent quality. The smoking process is still based on traditional principles and  
6 methods, but several parameters can influence the product before processing (rearing  
7 and slaughtering), during processing (salting and smoking) and afterwards during  
8 storage. The quality parameters texture, liquid holding capacity and colour of cold  
9 smoked salmon are affected by the processing as well as by the characteristics of the  
10 raw material. It is therefore important to apply a careful control during the  
11 manufacturing of cold smoked salmon, both with respect to the raw material and to the  
12 process.

13  
14 Keywords: smoked salmon, liquid loss, lipid content, fatty acid composition,  
15 processing, texture, colour

## **Introduction**

Cold smoked salmon is a product highly valued by the consumer and a commodity of economic importance on the world market. Production in Europe alone is more than 70.000 metric tonnes (FAO, 2003). However, during recent years, the salmon smoking industry has faced new problems such as high frequency of gaping (fig. 1), soft texture, low and uneven colour distribution and liquid leakage in the smoked product (fig. 2) (Espe et al., 2002; Rørå and Einen, 2003). As a result the image of cold smoked salmon is decline, resulting in considerable financial loss for the producers. The quality problem is underlined by several market surveys that have been published concerning retail samples of vacuum packed sliced cold salmon (Schubring, 2006; Séménou et al., 2007). Some products even show signs of spoilage both according to sensory analysis and chemical measurements (TVB-N) (EuroSalmon, 2003).

Several factors can influence the quality of the cold smoked product in the chain from live fish to final product, for example genetics and rearing conditions (density, feed, temperature, stress etc.) (Rasmussen, 2001) and slaughtering methods (Erikson et al., 1997; Olsen et al., 2006), as these parameters have an effect on the composition and texture of the raw material (Rørå et al., 1998). Furthermore, changes in the salting and smoking process and storing conditions combined with the requirement of a longer shelf life have an influence on the product quality.

Many studies have investigated those factors, which are important for rearing and the growth of salmon, but the quality of the final product is discussed only in very few of these studies (Rasmussen, 2001; Rasmussen et al., 2001; Johnston et al., 2007).

1 However, recent years four comprehensive studies concerning the product quality of  
2 cold smoked salmon have been published: A EU project “Improved quality of smoked  
3 salmon for the European consumer” (Eurosalmon, 2003) and three Ph.D. theses from  
4 Norway (Mørkøre, 2002; Rørå, 2003; Birkeland, 2004) have investigated several factors  
5 which affect quality. Based on the results from these studies, the literature and own  
6 studies, this paper reviews those factors which influence the quality of cold smoked  
7 salmon combined with methods used for the quality assessment in the industry. The  
8 focus is on the raw material, the process and the product parameters.

## 10 **Raw material**

11 Sigurgisladóttir et al., (1997) have reviewed methods to determine salmon quality,  
12 based on literature studies and interviews with companies throughout Europe. Those  
13 parameters of particular importance for the quality of salmon as a raw material were  
14 lipid content, composition and distribution of lipid, colour intensity and distribution of  
15 lipid in the fillets and texture.

## 17 **Influence of lipid content in the raw material on quality parameters**

18 Nowadays, farmed salmon is mainly used in the European smoking industry instead of  
19 traditional wild salmon. Farmed Atlantic salmon of market size has a lipid content  
20 between 6 % and 22 %, with 15-16 % as an average measured in the Norwegian quality  
21 cut (Rørå et al., 1998). High individual variations are seen, even for fish from the same  
22 batch there can be considerable variation (Bell et al., 1998; Rørå et al., 1998). In a study  
23 carried out at a commercial smoke house in Denmark it was found that the lipid content  
24 could vary between 8 % and 14 % in salmon purchased from the same batch. In Figure

3, the variation in lipid content in raw salmon is shown. Each week represents fish from the same batch and for each batch two boxes of salmon were investigated. Each box contained 5 to 7 salmon with 3-4 kg in size.

The lipids are not equally distributed in the fillet (Hardy and King 1989; Aursand et al., 1994; Refsgaard et al., 1998; Katikou et al., 2001). The highest lipid content is found in the dorsal lipid depot, the belly flap area and the red muscle, all of which contain three times as much lipid as the white muscle (Aursand et al., 1994).

The lipid content in farmed Atlantic salmon is correlated to several parameters such as the lipid content and the lipid composition of the feed (Lie et al., 1993; Bell et al., 1998; Hemre and Sandnes, 1999), annual variations (Aknes et al., 1986; Mørkøre and Rørvik, 2001) and the body weight of the fish (Storebakken et al., 1991; Shearer et al., 1994; Torrissen et al., 2001). Other parameters, e.g. genetic strain, environment and sexual maturation, may also affect the lipid content (Gjedrem, 1997). Thus, through the rearing regime it is possible to design a salmon with a particular lipid content.

An increased lipid content lead to a decrease in the smoked fillet yield (Rørå et al., 1998; Mørkøre et al., 2001; Birkeland et al., 2004a). However, the effect of high lipid content on the sensory parameters shows however contradictory results. Rørå et al., (1998) found that none of the sensory-evaluated parameters of smoked salmon were significantly correlated with the lipid content (range of 14 to 21 %) in raw salmon muscle. However, the total intensity of taste and the intensities of smoked, rancid and off-tastes increased with increasing fatness. Robb et al. (2002) found that an increased

lipid content (range of 2.9 to 10.7 %) led to a decreased firmness and increased oily taste in smoked salmon. Sheehan et al., (1996) found a significant effect of lipid content (range of 21 to 30 %) on the degrading of sensory attributes (oiliness, texture, colour and gaping) after 24 days after smoking. Mørkøre et al., (2001) found a positive correlation between sensory evaluated fatty texture and lipid content (range of 12 to 18 %) in the raw material, but many sensory attributes such as colour, flavour and texture were not affected by the lipid content as seen in Rørå et al., (1998). Thus, a lipid content seems to affect the sensory properties. The conflicting results can be due to the fact that the different smoked products have been stored for different times before sensory evaluation (5 to 24 days). In addition, the samples in one study (Robb et al., 2002) were frozen before the sensory evaluation

A high lipid content affects the colour of dry salted smoked fillets (Sheehan et al., 1996; Einen et al., 1999; Birkeland et al., 2003) as fillets with a high lipid content are more light and yellow than fillets with a low lipid content (Birkeland et al., 2003). In the study by Birkeland et al., (2003) the redness was not affected by the lipid content although that has been seen by Sheehan et al., (1996) and Einen et al., (1999). Different salting methods were used in the three studies (dry salting, brine salting and injection salting), and application of different salting methods affects the colour (Birkeland, 2004), which might explain the different results regarding the redness of the smoked fish. High lipid content has been reported to lead to a decreased liquid holding capacity in smoked salmon (Mørkøre et al., 2001; Birkeland et al., 2004a).

Information about the lipid content is of importance for the industry in order to control

the production quality. The lipid content is traditionally determined by time-consuming chemical extraction methods. However within recent years more rapid methods have been investigated. Computerised X-ray tomography (CT) is based on X-rays and is a non-destructive method for the determination of lipid contents and lipid distribution in rainbow trout and salmon (Mørkøre et al., 2001; 2002; Nanton et al., 2007). However, high investment costs, size and complexity make the instrument less suitable for use in the fish industry. Another new method for rapid measurement of lipid content in live or slaughtered Atlantic salmon is a mobile low-field nuclear magnetic resonance (NMR), which has been used to determine lipid contents in live fish (Veliyulin et al., 2005). The mobile NMR spectrometer has potential for implementation in connection with on- or at-line grading in the processing plant.

To conclude, knowledge or measurement of the lipid content is of importance for the industry so as to be able to grade the raw material in order to assure a consistent quality. The lipid content in fillets affects yield, colour, liquid holding capacity and often sensory attributes. High variation in lipid contents can be found for farmed salmon from the same production batch (even from the same box) and the lipid content is not equally distributed in the fillet. However, the lipid content is influenced by several controllable parameters during rearing, so in the future it may be possible to design salmon with a desired range of lipid.

### **Influence of the fatty acid composition in the raw material on quality**

Fish is a health-promoting food due to the high contents of n-3 polyunsaturated fatty acids (PUFA) (Kris-Etherton et al., 2002). The long chain n-3 polyunsaturated fatty acid



1 docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) in  
2 fish are recognized to contribute to the positive effect of particular fatty fish. It is  
3 therefore essential that farmed salmon have the level of n-3 expected by the consumer.  
4 Fish meal and fish oil have traditionally been used as the major raw material ingredients  
5 in salmon feeds, and a high amount of the total global fish oil is used for fish feed  
6 production. However the increase in the production of farmed fish has led to a reduced  
7 availability of fish oil and to an increased price of fish oil. Vegetable oils are thus  
8 partially replacing fish oil in the diet of farmed salmonids (Sargent et al., 2001; Bell et  
9 al., 2001; 2002). Alternative oil sources are soybean, rapeseed and sunflower oil. The  
10 content of fatty acid in the lipid of the diets will be reflected in the fish muscle (Hardy et  
11 al., 1987; Bell et al., 2001; 2002). The fatty acid profile of the muscle does not change  
12 significantly during smoking (Rørå et al., 2003; Regost et al., 2004). Thus, the fatty acid  
13 profile of the raw material seems to be reflected in the smoked fillets.

14  
15 Alternative lipid sources can replace part of the fish oil in the diet without sacrificing  
16 growth or reproduction (Bell et al., 2001, 2002; Grisdale-Helland et al., 2002). The  
17 effects of vegetable oil on pigmentation, texture, storage stability and sensory  
18 characteristics are dependent on the level of substitutions. In recently published studies  
19 (Bjerkeng et al., 1997; Rørå et al., 2003; Torstensen et al., 2005), it was shown that  
20 vegetable oil (either pure soy oil or a mixture of rapeseed, linseed and palm oil) can be  
21 used in the grow-out phase without changing the quality in terms of texture and liquid  
22 holding capacity. However, a total substitution of the fish oil by soybean oil in diets for  
23 salmon affected the muscle colour and the fatty acid profile (Rørå et al., 2005a). When  
24 the vegetable oil is substituted with pure fish oil in the finishing growth phase (5

months), sensory evaluation by a trained panel shows only minor differences between dietary groups. But prior to the finishing period, the fish oil group was preferred to the fish fed on 100% vegetable oil or a mixture of oils (Torstensen et al., 2005).

The majority of studies find no significant effect of dietary lipid source on flesh texture (Rørå et al., 2003; Bell et al., 2001). The effect of fatty acid composition on the liquid holding capacity has been studied and contradictory results have been found. Rørå et al. (2005b) found a significant effect of dietary treatment on smoked fillet as the fish fed on a diet with soybean oil had a lower liquid holding capacity than fish fed on a diet consisting of fish oil. However another study, Rørå et al. (2003), found no effect of dietary oil sources on the liquid holding capacity in fresh and smoked salmon. The fatty acid profile of the muscle does not affect selective leakage of specific fatty acids (Rørå et al., 2003).

To conclude, the fatty acid composition of the feed is reflected in the fatty acid composition of the muscle and the fatty acid profile of the raw material seems to be reflected in the smoked fillets. Alternative lipid sources can replace part of the fish oil in the diet without affecting growth or reproduction. The effects of vegetable oil on pigmentation, texture, storage stability and sensory characteristics are dependent on the level of substitutions. Contradictory results have been found according to the effect of fatty acids on liquid holding and colour. However, it is important to be aware of that the qualities, the health of the fish and in healthy image of salmon due to the content of n-3 fatty acids are not degraded by the inclusion of vegetable in the diet. By using pure fish oil in the finishing growth phase, it is possible to obtain to the optimal fatty acid

composition.

### **The cold smoking process**

Cold smoked salmon is considered a lightly preserved fish product with a salt content ranging from 3.5 to 6.0 % in the water phase (Hansen et al., 1996), a water content between 63 and 70 % (Espe et al., 2004; Cardinal et al., 2004) and a pH between 5.8 and 6.3 (Hansen et al., 1995).

Cold smoked salmon is produced by a large number of smoke houses using traditional or more modern automated production methods. The cold smoking process has developed from a simple process to a modern industrial process. However the fundamental principles in the smoking technique are still used by the commercial smoke houses. Salting, drying and smoking are used in combination to preserve the fish. Due to the development of more modern packaging technologies and refrigeration the preservation part of the smoking process has become less important. Thus, the extent of salting, drying and smoking of the products has been reduced. Traditionally dry salting has been used as the salting method, but recently injection salting has been adapted by the producers of cold smoked salmon. For smoking modern forced convection ovens are used, and the temperature, air velocity, air-convection direction and smoke-generator temperature can be controlled during smoke processing (Birkeland, 2004). In Figure 4, a flow chart for the cold smoking process for salmon is shown from the slaughtering to the final product.

In addition to the steps mentioned in Figure 4, freezing can be included in different

steps. The smoke houses buy both fresh and frozen raw fish. Smoke houses may choose to freeze their fish either before or after processing due to logistics, prices, etc. Very often the smoked fillet is frozen short-term to make it easier to slice the smoked fillet (Rørå, 2003). The smoked product might also be stored frozen before being transported to the retail store in order to ease logistics. The smoked product may also be frozen by the consumer before consumption.

The effects of freezing have been investigated in a few studies. The freezing may influence the salt uptake before smoking. Cardinal et al., (2001) found that the salt content in smoked products was slightly higher when the raw material (as whole) had been frozen compared to fresh salmon and this was explained by a slight modification of the cell structure by freezing which increases the diffusion of salt. Rørå and Einen (2003) found that the astaxanthin level was reduced both by freezing before and after smoking. Cardinal et al., (2001) found that the salmon was more yellow (higher  $b^*$  value) when the salmon had been frozen. Freezing also affects the muscle structure and thus the texture. Freezing before smoking causes softer texture of smoked fish (Dunajski, 1979; Rørå and Einen, 2003), and freezing of fillets before smoking increase the incidence of gaping in the smoked fillets. Rørå and Einen (2003) concluded that freezing after smoking led to fewer changes in quality parameters of cold smoked salmon than freezing before smoking. More research is needed to explore the effect of freezing on the quality of cold smoked salmon.

The techniques used for salting, drying, smoking and storing can affect both the yield and the general quality. Salting provides the desired firm texture and the salty taste of

the flesh (Doe et al., 1998). Salting also preserves and increases the shelf life of the product as salting results in lower water activity, thereby lowering microbial activity. The recommended critical limit for salt content in chilled vacuum-packed cold smoked salmon is 3.5 % salt in the water phase to prevent growth and toxin production by *Clostridium botulinum* during chill storage. Different salting methods are applied in the industry e.g. dry, brine or injection salting.

By adapting the injection salting method, production time is saved and a higher processing yield is obtained (Birkeland et al., 2003, 2004ab) compared to the dry salting and brine salting methods. The injection salting method leads to products that differ in characteristics compared to dry salted smoked products (Birkeland et al., 2004ab). Higher gaping score and softer texture for salt injected salmon fillets compared to dry salted salmon fillets have been reported (Birkeland et al., 2004a). The observed differences in the textural characteristics could be due to different water contents in smoked fillets caused by the different salting methods applied (Jittinandana et al., 2002). During dry salting there is a loss of water while during injection salting water is injected into the muscle tissue (Birkeland et al., 2003).

Birkeland et al., (2004b) found that the dry salting technique affected the variation in colour characteristic more than the injection salting technique, which indicated that the injection salting technique give a more homogenous smoked product with respect to colour characteristics than that obtained after dry salting. Birkeland and Bjerkeng (2005) found that the salting temperature and salting time of brine or dry salting had substantial effects on the colour of cold-smoked Atlantic salmon. The samples which

were brine salted or dry salted at low temperature (4°C) were significantly more light and yellow compared to fillets salted at 10°C. Upon increasing the salting time from 6 hour to 12 hours the fillets became less light and red. Further increase of the brining time from 12 hours to 24 hours did not cause any significantly effects on surface colouration of the smoked fillets. The colour parameters of smoked fillets were more affected by the brine concentration than by brining time and temperature. The fillets salted in a brine concentration of 50 % saturated salt solution were significantly less red than fillets salted in a brine concentration of 100 %. The brine concentration did not affect the lightness and yellowness of the smoked fillets.

Birkeland and Bjerkeng (2005) found that increasing salting temperature (4 to 12°C) significantly increased the weight loss by 1.5 % units and increased salting time (6 hours vs. 24 hours) significantly increased the weight loss by 3.2 % units. Other studies (Cardinal et al., 2004; Mørkøre et al., 2001) have found similar weight losses following dry salting.

The yield after smoking is an important factor for financial success in the smoking industry. The smoking temperature affects the yield of smoked Atlantic salmon fillets, but the results are contradictory. Sigurgisladóttir et al., (2000a, 2000b) and Cardinal et al., (2001) found higher smoking yields in Atlantic salmon fillets smoked at a temperature of 30°C compared to 20°C. Birkeland et al., (2003, 2004a) found that smoking at a high temperature (30°C) compared to a low temperature (20°C) decreased the smoking yield. Increasing yield of smoked Atlantic salmon fillets with increasing fillet lipid content has been reported (Cardinal et al., 2001; Torrissen et al., 2001). The

1 differences in yield after smoking between fillets with high lipid contents may be  
2 explained by reduced dehydration during the processing of fillets with a high lipid  
3 content (Sigurgisladóttir et al., 2000a; Torrissen et al., 2001). The smoking temperature  
4 affects the texture as higher firmness and elasticity have been reported for fillets  
5 smoked at 29.9°C compared to fillets smoked at 21.5°C (Rørå et al., 2005b). However,  
6 the temperature explained less than 35% of the variation in the textural properties of the  
7 smoked fillets.

8  
9 To conclude, the recommended salt content of chilled vacuum-packed cold smoked  
10 salmon is 3.5 % salt in the water phase. Different salting methods are applied in the  
11 industry e.g. dry, brine or injection salting. Several factors can affect the uptake and  
12 distribution of salt in salmon fillets. The highest yield is obtained after injection salting,  
13 while injection salting gives a softer texture and gaping in the smoked fillets compared  
14 with dry salting and brine salting. Dry salting affects the colour parameter more than the  
15 injection salting. Brine or dry salting at low temperature gives fillets which are  
16 significant lighter and more yellow in colour compared to fillets salted at 10°C. Upon  
17 increasing the salting time from 6 to 12 hours the smoked fillets became darker and  
18 redder. The colour parameters of smoked fillets were affected more by the brine  
19 concentration than by brining time and temperature. Fillets processed according to dry  
20 salting were firmer and more elastic than fillets which were processed according to  
21 injection-salting.

22  
23 The conditions applied during smoking affect the colour parameter and texture. A high  
24 smoking temperature gives a firmer and more elastic texture and a reduced redness in

smoked salmon compared with smoking at low temperature. A long smoking time decreases the yield after smoking.

## **Quality parameters of cold smoked salmon**

### **Texture in cold smoked salmon**

The texture of fish is an important quality characteristic. Fillets with a soft texture and/or gaping can cause considerable financial losses due to downgrading of the product (Lavéty et al., 1988; Michie, 2001). The term gaping is used to describe the gaps, tears or slits that are found in post mortem fish flesh (Bremner, 1999), and muscle gaping occurs when the connective tissue fails to hold the blocks of muscle together (Lavéty et al., 1988; Love, 1988). Gaping in salmon makes the flesh unsuitable for the production of sliced products. Products with gaping will be less presentable, and since the flesh is soft, the texture will be less acceptable (Bremner, 1999).

The texture of fish muscle is influenced by season (Mørkøre and Rørvik, 2001), the collagen content (Sato et al., 1986; Bremner, 1992), post mortem factors, pH (Einen et al., 1999) and the lipid content and the distribution of muscle lipid (Regost et al., 2004).

The texture of fish muscle and the degree of gaping are influenced by the collagen content. High collagen content is associated with firmer texture and less incidence of gaping (Sato et al., 1986; Hatae et al., 1986). Raw fish flesh from most fish species softens after a few days of chilled storage (Sato et al., 1991). Histological studies have shown that the rapid softening of fish flesh is caused by a weakening of connective tissue resulting from a disintegration of thin collagen fibrils (Hallett and Bremner, 1988;



1 Ando et al., 1991; Ando et al., 1992).

2  
3 During the cold smoking process, the texture of the fish fillet will change. The  
4 processing parameters that affect the textural characteristics are the processing  
5 temperature, salt/ionic strength and pH, since these have an influence on the structure  
6 and conformation of the myofibrillar and connective tissue proteins (Dunajski 1979).

7 Cold smoked salmon products are firmer and more elastic compared to raw,  
8 unprocessed fillets (Sigurgisladóttir et al., 1999; 2000a).

9  
10 The higher lipid content has been claimed to affect the texture (Einen et al., 1999;  
11 Sheehan et al., 1996). Softness and gaping have been associated with high lipid content  
12 in farmed salmon (Sheehan et al., 1996), which can result in difficulties when slicing  
13 the smoked fish (Eckhoff et al., 1998). However, Andersen et al., (1997) found that the  
14 fillet lipid content did not affect muscle gaping in raw fillets of rainbow trout. Birkeland  
15 et al., (2004a) found no evidence of lipid content in fillets affecting the gaping scores in  
16 smoked Atlantic salmon following dry- or injection salting, whereas Sheehan et al.,  
17 (1996) found that gaping was most severe in smoked fillets which had been fed on a  
18 high lipid diet. In the study by Sheehan et al., (1996), the fillets were chilled stored  
19 (4°C) for 24 days, while the effect of chill storage was not investigated in the study by  
20 Birkeland et al., (2004a). Gaping is influenced by the salting method as more muscle  
21 gaping was found in injection-salted than dry salted fillets (Birkeland et al., 2004a). The  
22 texture properties of smoked fillets are affected by fillet lipid content. Birkeland et al.,  
23 (2004a) found that the fillets with low lipid contents (16.6 %) were significantly more  
24 elastic than the fillets with high lipid content (20.0 %) and fillets with low lipid content

were firmer than fillets with high lipid content. Sheehan et al., (1996) found that smoked fillets fed with a low lipid diet (21 %) were significantly softer than fillets of fish fed with a medium (25 %) or a high lipid diet (30 %).

To conclude, the texture of fish changes during the cold smoking process and becomes more firm and elastic. The texture is influence by the lipid content and collagen content as well as post mortem factors and processing. Contradictory results have been found regarding the effect of lipid content on gaping.

#### **Liquid holding capacity in smoked salmon**

The liquid holding capacity of fish muscle is of major importance with respect to both commercial value and consumer acceptance (Elvevoll et al., 1996). A reduced liquid holding capacity makes the fillets more prone to liquid leakage during smoking or afterwards during slicing and vacuum storage. Accumulated liquid leakage in vacuum-packaged smoked salmon has a negative effect on the product appearance (Birkeland, 2004). In addition, the smoked products may be difficult to slice due to excessive oiliness (Bell et al., 1998).

The liquid holding capacity is defined as the ability of a given structure to prevent water and/or lipids from being released from the structure, and it refers therefore to both water and lipid holding capacity (Hermansson, 1986).

The liquid holding capacity of fish muscle can be influenced by salt concentration (Ofstad et al., 1996), pH (Regenstein et al., 1984; Ofstad et al., 1995, 1996), storage

conditions (Mackie, 1993; Nilsson and Ekstrand, 1994) and heat (Ofstad et al., 1993, 1995, 1996), all of which influence the structure and conformation of myofibrillar and connective tissue proteins.

.  
Birkeland et al., (2004a) found that the mean percentages of lipid and water in the liquid loss in smoked fillets were significantly affected by processing. A gentle smoking method, including dry salting, showed more lipid in the liquid loss than fillets from a tougher smoking method, including injection salting. This may be due to the extraction of water during dry-salting, whereas the injection method includes injection of significant amounts of water into the muscle. The liquid loss was also higher for the dry salted samples than for the injected salted samples.

The liquid holding capacity is higher for raw samples than for smoked samples (Løje et al. submitted; Birkeland et al., 2004a). However higher liquid holding capacity in smoked fish compared to raw salmon has also been reported (Rørå et al., 2003) The liquid holding capacity is affected by the lipid content in the fillet, and reduced liquid holding capacity has been found in smoked salmon with high lipid contents (Mørkøre et al., 2001; Birkeland et al., (2004a).

Storage time and storage temperature affected the liquid holding capacity of smoked salmon as the liquid holding capacity decreased when the storage temperature increased as well as when the storage time increased (Rørå et al., 2003). The decrease was more pronounced when the two conditions were combined, whereby a liquid loss of 4.4 % was found after 15 days of storage at 14°C. During chill storage at 2°C for 20 days of

the smoked salmon samples, a decrease in liquid holding capacity was observed (Løje et al., submitted).

Different studies of liquid holding capacity in salmon and rainbow trout have used different methods and different experimental conditions, and this makes it difficult to compare the results. Centrifugation methods have been used in several studies. However this method is only feasible for use in a laboratory. Thus, there is a need for a reliable method to determine liquid holding capacity which can be used in a production plan.

To conclude, the liquid holding capacity is an important parameter as a high liquid loss and thereby a low liquid holding capacity can lead to a downgrading of the product. The liquid holding capacity of smoked salmon is affected by salting method and conditions used for salting as well as storage time and storage temperature after smoking. Other parameters affecting the liquid holding capacity are pH, storage, heat and salt. The lipid content also influence the liquid holding capacity as lower liquid holding capacity has been found in fillets with a high lipid content.

### **Colour of cold smoked salmon**

The colour of a product gives the first impression, and if the consumer dislikes the colour, then the other quality parameters such as texture or flavour are not likely to be judged at all (Francis, 1995). Thus, the colour of the flesh is among the most important quality characteristics of salmon (Sigurgisladóttir et al., 1997). The red colour of salmon flesh is due to carotenoids, and farmed salmon derive the colour from the carotenoid astaxanthin, which is added to the feed (Skrede and Storebakken, 1986a; Bjerkeng,

2004). Another carotenoid, canthaxanthin, can also be used either alone or in combination with astaxanthin. However, restrictions on the use of canthaxanthin have been introduced by the EU (Bjerkeng, 2004).

Problems related to discolouration are occasionally observed in salmon and salmonid fish species. Several kinds of colour deficiencies exist and include poor pigmentation, discolouration, partly or complete depigmentation and zebra stripes (Torrissen, 1995). Several factors can influence the colour e.g. the concentration and type of the pigment in the feed (Skrede and Storebakken, 1986b; Bjerkeng, 2000), dietary lipid level (Regost et al., 2001), the size of the fish, the stage of sexual maturity (Aknes et al., 1986; Bjerkeng et al., 1992), the lipid content in the fish (Rørå et al., 1998) and processing (Birkeland and Bjerkeng, 2005). The smoking process causes changes in the colour. A general trend is that cold smoked fillets are less red and less light, but more yellowish compared to the raw material (Rørå et al., 1998; Cardinal et al., 2001; Birkeland *et al.* 2004).

The colour can be estimated or measured by using non-destructive instrumental methods. One of the most common method is the  $L^*a^*b^*$  system. The colour of salmon is then characterised by the parameters  $L^*$ ,  $a^*$  and  $b^*$  values. The  $L^*$  variable represent lightness ( $L^* = 0$  for black,  $L^* = 100$  for white), the  $a^*$  scale represents the intensity in red and the  $b^*$  scale represents the intensity in yellow. The Minolta Chroma Meter is one of the most commonly used instruments. The colour of the fillet can also be measured by a Roche colour score card. The fillet or cutlet is compared with the colour card and the colour estimated according to the scale on the card. The Roche colour card

is commonly used by fish farms, slaughterhouses and processing plants in Europe and U. S. (Sigurgisladdottir et al., 1997). The card is inexpensive compared to instrumental measurements and simple in use. Recently a computer vision method was used to evaluate the colour of Atlantic salmon and thereby to sort the fillet in a fast and non-destructive manner (Misimi et al., 2007).

To conclude, the colour of cold smoked salmon is an important parameter as it is the impression that the consumer has of the product. The colour is affected by the processing and cold smoked products are in general less red and light, and more yellowish than raw samples.

## **Conclusion**

In recent years, several studies of cold smoked salmon have been published showing a small but significant influence of breeding and processing parameters (Birkeland, 2004). These studies carried out under experimental conditions show that salmon constitutes a fairly robust raw material for cold smoking.

The lipid content shows considerable variation. High variation between individual fish in the same batch has been reported. In addition the lipid content is not equally distributed in the fillet. The lipid content can be influenced by several parameters due to rearing, genetics etc. The lipid content in fillet affects yield, colour, liquid holding capacity, and have some effect on sensory attributes. Thus, knowledge of the lipid content is of importance for the industry to be able to assure an even quality. The fatty acid composition of the feed is reflected in the fatty acid composition of the muscle and

1 the fatty acid profile of the muscle does not change significantly during smoking.  
2 Alternative lipid sources can replace part of the fish oil in the diet without affecting  
3 growth or reproduction. The effects of vegetable oil on pigmentation, texture, storage  
4 stability and sensory characteristics are dependent on the level of substitutions.  
5 Contradictory results have been found regarding the effect of fatty acids on liquid  
6 holding and colour. Fish is recognized as a health-promoting food due to the high  
7 content of n-3 polyunsaturated fatty acids and to keep this health image it is important  
8 that the have a high level of these fatty acids. This can be obtained by using pure fish oil  
9 in the growth out phase.

10  
11 The smoking process is still based on traditional principles and methods. However  
12 modern full automatical ovens are now available, where the process parameters can be  
13 controlled. It is also possible to use electrostatic smoking or smoke-curing with liquid  
14 smoke condensates. For salting different methods are available. However the main steps  
15 in the cold smoking process are still salting, drying and smoking. The recommended salt  
16 content of chilled vacuum-packed cold smoked salmon is 3.5 % salt in the water phase.  
17 Different salting methods are applied in the industry e.g. dry, brine or injection salting.  
18 Several factors can affect the uptake and distribution of salt in salmon fillets. The  
19 applied conditions during smoking affect the colour parameter and texture. It seems like  
20 a high smoking temperature gives a firmer and more elastic texture and a reduced  
21 redness in smoked salmon compared with smoking at low temperature. A long smoking  
22 time decreases the yield after smoking.

23  
24 The quality parameters e.g. texture, liquid holding capacity and colour of cold smoked

salmon as influenced by several factors. The texture of fish changes during the cold smoking process and becomes firmer and more elastic. The texture is influence by the lipid content and collagen content as well as post mortem factors and processing. The liquid holding capacity is an important parameter as a high liquid loss due to low liquid holding capacity can lead to downgrading of the product. The liquid holding capacity of smoked salmon is affected by the salting method and conditions used for salting and smoking as well as storage time and storage temperature after smoking. Other parameters affecting the liquid holding capacity are pH, storage, heat and salt. The lipid content also influences the liquid holding capacity as lower liquid holding capacity has been found in fillets with a high lipid content. The colour of cold smoked salmon is affected by the processing and cold smoked products are in general less red and light, and more yellowish than raw samples.

## REFERENCES

- Aknes, A., Gjerde, B., and Roald, S.O. 1986. Biological, chemical and organeleptic changes during maturation of salmon (*Salmo salar*). *Aquaculture* 53: 7-20.
- Andersen, U., Thomassen, M.S., and Rørå, A.M.B. 1997. Texture properties of farmed rainbow trout (*Oncorhynchus mykiss*): Effects of diet, muscle fat content and time of storage on ice. *J. Sci. Food Agric.* 74: 347-353.
- Ando, M., Toyohara, H., Shimizu, Y., and Sakaguchi, M. 1991. Post-mortem tenderization of fish muscle proceeds independently of resolution of rigor mortis. *Bull Jpn. Soc. Sci. Fish* 57 (6): 1165-1169.



1  
2 Ando, M., Toyohara, H., and Sakaguchi, M. 1992. Three-dimensional structure of  
3 collagen fibrillar network of pericellular connective tissue in association with firmness  
4 of fish muscle. Bull. Jpn. Soc. Sci. Fish 58 (7): 1361-1364.

5  
6 Aursand, M., Blevik, B., Rainuzzo, J.R., Jørgensen, L., and Mohr, V. 1994. Lipid  
7 distribution and composition of commercially farmed Atlantic salmon (*Salmo salar*). J.  
8 Sci. Food Agric. 64: 239-248.

9  
10 Bell, J.G., McEvoy, J., Webster, J.L., McGhee, F., Millar, R.M., and Sargent, J.R. 1998.  
11 Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo*  
12 *salar*). J. Agric. Food Chem. 46: 119-127.

13  
14 Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., and Sargent, J.R.  
15 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo*  
16 *salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr.  
17 131: 535-1543.

18  
19 Bell, J.G., Henderson, J., Tocher, D.R., McGhee, F., Dick, J.R. Porter, A., Smullen,  
20 R.P. and Sargent, J.R. 2002. Substituting fish oil with crude palm oil in the diet of  
21 Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty  
22 acid metabolism. J. Nutr. 132: 220-230.

23  
24 Birkeland, S. 2004. Cold-smoking of Atlantic salmon (*Salmo salar*) – Influence of

processing conditions and raw material characteristics on quality and yield. Doctor Thesis, Department of Animal and Aquacultural Sciences, Agricultural University of Norway.

Birkeland, S., and Bjerkeng, B. 2005. The quality of cold-smoked Atlantic salmon (*Salmo salar*) as affected by salting method, time and temperature. Int. J. Food Sci. Technol. 40: 963-976.

Birkeland, S., Rørå, A.M.B., Skåra, T., and Bjerkeng, B. 2003. Product yield and gaping in cold-smoked Atlantic salmon (*Salmo salar*) fillets as influenced by different injection-salting techniques. J. Food Sci. 68(5): 1743-1748.

Birkeland, S., Røra, A.M.B., Skåra, T., and Bjerkeng, B. 2004a. Effects of cold smoking procedures and raw material characteristics on product yield and quality parameters of cold smoked Atlantic salmon (*Salmo salar* L) fillets. Food Res. Internat. 37: 273-286.

Birkeland, S., Haarstad, I., and Bjerkeng, B. 2004b. Effects of salt-curing procedure and smoking temperature on astaxanthin stability in smoked salmon. J. Food Sci. 69: 198-203.

Bjerkeng, B. 2000. Carotenoid pigmentation of salmonid fishes – recent progresss. In: *Avances en Nutrición Acuicola V. Memorias del V Simposium Internacional de Nutrición Acuicola* (Eds.: Cruz-Suárez, L. E., Ricque-Marie, D. Tapia-Salazar, M., Olvera-Novoa, M. A., and Civera-Cerecedo, R.). 19-22. November, Mérida, Yucatán.

pp 71-89.

Bjerkeng, B. 2004. Colour deficiencies in cold-smoked Atlantic salmon. I Dybden, 23 (2): 401-413.

Bjerkeng, B., Storebakken, T. and Liaaen-Jensen, S. 1992. Pigmentation of rainbow trout from start feeding to sexual maturation. Aquaculture 108: 333-346.

Bjerkeng, B., Refstie, S., Fjalestad, K.T., Storebakken, T., Rødbotten, M., and Roem, A.J. 1997. Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. Aquaculture 157: 297-299.

Bremner, H.A. 1992. Fish flesh structure and the role of collagen - its post-mortem aspects and implications for fish processing. In *Quality assurance in the fish industry* (Eds.: Huss, H. H., Jakobsen, M., Liston, J.). Amsterdam, London, New York, Tokoy, Elsevier. pp 41-62.

Bremner, H.A. 1999. Gaping in fish flesh. In: *Extracellular matrix of fish and shellfish* (Eds: Sato, K., Sakaguchi, M. and Bremner, H. A.). Research signpost, Trivandrum, India, Chapter VII, pp 81-93.

Cardinal, M., Knockaert, C., Torrissen, O.J., Sigurgisladottir, S., Mørkøre, T., Thomassen, M.S., and Vallet J.L. 2001. Relation of smoking parameters to the yield,

- 1 colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). Food Res.  
2 Internat. 34: 537-550.  
3
- 4 Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J.L., and Leroi,  
5 F. 2004. Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from  
6 European market and relationships with chemical, physical and microbiological  
7 measurements. Food Res. Internat. 37: 181-193.  
8
- 9 Doe, P., Sikorski, Z., Haard, N.F., Olley, J., and Pan, B.S. 1998. Basic principles: In:  
10 *Fish drying and smoking. Production and quality* (Ed.: Doe, P.). Lancaster,  
11 Pennsylvania, USA: Technomic Publishing Company Inc. pp 13-45.  
12
- 13 Dunajski, E. 1979. Texture of fish muscle. J. Texture Stud., 10: 301-318.  
14
- 15 Eckhoff, K.M., Aidos, I., Hemre, G-I., and Lie, Ø. 1998. Collagen content in farmed  
16 Atlantic salmon (*Salmo salar*, L.) and subsequent changes in solubility during storage  
17 on ice. Food Chem. 62 (2): 197-200.  
18
- 19 Einen, O., Mørkøre, T., Rørå, A.M.B., and Thomassen, M.S. 1999. Feed ration prior to  
20 slaughter - a potential tool for managing product quality of Atlantic salmon (*Salmo*  
21 *salar*). Aquaculture 178: 149-169.  
22
- 23 Elvevoll, E.O., Sørensen, N. K., Østerud, B., Ofstad, R., and Martinez, I. 1996.  
24 Processing of marine foods. Meat Sci. 43: 265-275.

Erikson, U., Sigholt, T., and Seland, A., 1997. Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 149: 243–252.

Espe, M., Nortvedt, R., Lie, Ø., and Hafsteinsson, H. 2002. Atlantic salmon (*Salmo salar* L.) as raw material for the smoking industry. II: Effects of different smoking methods on losses of nutrients and on the oxidation of lipids. Food Chem. 77 (1): 41-46.

Espe, M., Ruohonen, K., Bjørnevik, M., Frøyland, L., Nortvedt, R., and Kiessling, A. 2004. Interactions between ice storage time, collagen composition, gaping and textural properties in farmed salmon muscle harvested at different times of the year. Aquaculture 240: 489-504.

EuroSalmon. 2003. Improved quality of smoked salmon for the European consumer.

<http://www.mmedia.is/matra/eurosalmon/>

FAO Yearbook 2003, Fisheries Statistics, Commodities 2001, vol 93, pp 143.

Francis, F.J. 1995. Quality as influenced by color. Food Qual. Pref. 6: 149-155.

Gjedrem, T. 1997. Flesh quality improvement in fish through breeding. Aquac. Int. 5: 197-206.

1  
2 Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S.J., Sandberg, M.  
3 G., Standal, H., and Røsjø, C. 2002. Influence of high contents of dietary soybean oil on  
4 growth, feed utilization, tissue fatty acid composition, heart histology and standard  
5 oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures.  
6 Aquaculture 207: 311-329.

7  
8 Hallett, I.C. and Bremner, H.A. 1988. Fine structure of the myocommata-muscle fibre  
9 junction in hoki (*Macruronus novaezelandiae*). J. Sci. Food Agric. 44: 245-261.

10  
11 Hansen, L.T., Gill, T., and Huss, H.H. 1995. Effects of salt and storage temperature on  
12 chemical, microbiological and sensory changes in cold-smoked salmon. Food Res.  
13 Internat. 28 (2): 123-130.

14  
15 Hansen, L.T., Gill, T., Rontved, S.D., and Huss, H.H. 1996. Importance of autolysis and  
16 microbiological activity on quality of cold-smoked salmon. Food Res. Internat. 29 (2):  
17 181-188.

18  
19 Hardy, R.W., and King, I.B. 1989. Variation in n-3 fatty acid content of fresh and  
20 frozen salmon. Omega 3 News 4: 1-4.

21  
22 Hardy, R.W., Scott, T.M., and Harrell, L.W. 1987. Replacement of herring oil with  
23 menhaden oil, soybean oil or tallow in the diets of Atlantic salmon raised in marine net-  
24 pens. Aquaculture 65: 267-277.

Hatae, K., Tobimatsu, A., Takeyama, M., and Matsumoto, J. J. 1986. Contribution of the connective tissue on the texture difference of various fish species. Bull. Jpn. Soc. Sci. Fish 52 (11): 2001-2007.

Hemre, G.I., and Sandnes, K. 1999. Effect of dietary lipid level on muscle composition in Atlantic salmon (*Salmo salar*). Aquac. Nutr. 5: 9-16.

Hermansson, A. M. 1986. Water- and fat holding. In: *Functional properties of food macromolecules* (Eds. Mitchell, J. R. and Ledward, D. A.). London: Elsevier Ltd. pp 273-314.

Jittinandana, S., Kenney, P. B., Slider, S. D. and Kiser, R. A. 2002. Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. J. Food Sci. 67 (6): 2095-2099.

Johnston, I.A., Bickerdike, R., Li, X., Dingwall, A., Nickell, D., Alderson, R., and Campbell, P. 2007. Fast growth was not associated with an increased incidence of soft flesh and gaping in two strains of Atlantic salmon (*Salmo salar*) grown under different environmental conditions. Aquaculture 265: 148-155.

Katikou, P., Hughes, S.I., and Robb, D.H.F. 2001. Lipid distribution within Atlantic salmon (*Salmo Salar*) fillets. Aquaculture 202: 89-99.

- 1 Kris-Etherton, P.M., Harris, W.S., and Appel, L.J. 2002. Fish consumption, fish oil,  
2 omega-3 fatty acids and cardiovascular diseases. Am. Heart Assoc. Circu. 106: 2747-  
3 2757.
- 4
- 5 Lavéty, J., Afolobi, O.A., and Love, R.M. 1988. The connective tissue of fish. IX.  
6 Gaping in farmed species. Int. J. Food Sci. Technol. 23: 23-30.
- 7
- 8 Lie, Ø., Sandvik, A., and Waagø, R. 1993. Influence of dietary fatty acids on the lipid  
9 composition of lipoproteins in farmed Atlantic salmon (*Salmo salar*). Fish Physio.  
10 Biochem. 12: 249-260.
- 11
- 12 Love, R.M. 1988. The food fishes: Their intrinsic variation and practical implication.  
13 Farrand Press, London.
- 14
- 15 Mackie, I.M., 1993. The effect of freezing on flesh proteins. Food Rev. Int. 9: 575-610.
- 16
- 17 Michie, I. 2001. Causes of downgrading in the Salmon farming industry. In: *Farmed*  
18 *fish quality* (Eds.: Kerstin S.C, and Warriss, P. D.). Bristol, UK: Fishing News Books,  
19 Blackwell Science Ltd. pp 129-136.
- 20
- 21 Misimi, E., Mathiassen, J.R., and Erikson, U. 2007. Computer vision- based sorting of  
22 Atlantic salmon (*Salmo salar*) fillets according to their color level. J. Food Sci. 72: 30-  
23 35.
- 24



1 Mørkøre, T. 2002. Texture, fat content and product yield of salmonids. Doctor Thesis,  
2 Department of Animal and Aquacultural Sciences, Agricultural University of Norway.

3  
4 Mørkøre, T. and Rørvik, K.A. 2001. Seasonal variations in growth, feed utilisation and  
5 product quality of farmed Atlantic salmon (*Salmo salar*) transferred to seawater as 0 +  
6 smolts or 1+ smolts. Aquaculture 199: 145-157.

7  
8 Mørkøre, T., Vallet, J.L., Cardinal, M., Gómez-Guillén, M.C., Montero, P., Torrissen,  
9 O.J., Nordtvedt, R., Sigurgisladottir, S., and Thomassen, M.S. 2001. Fat content and  
10 fillet shape of Atlantic salmon: Relevance for processing yield and quality of raw and  
11 smoked products. J. Food Sci. 66: 1348-1354.

12  
13 Mørkøre, T., Hansen, A.Å., Unander, E., and Einen, O. 2002. Composition, liquid  
14 leakage and mechanical properties of farmed rainbow trout: Variation between fillets  
15 sections and the impact of ice and frozen storage. J. Food Sci. 67: 1933-1938.

16  
17 Nanton, D.A., Vegusdal, A., Rørå A.M.B., Ruyter, B., Baeverfjord, G., and Torstensen,  
18 B.E. 2007. Muscle lipid storage pattern, composition, and adipocyte distribution in  
19 different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil.  
20 Aquaculture 265: 230-243.

21  
22 Nilsson, K., and Ekstrand, B. 1994. Refreezing rate after glazing affects cod and  
23 rainbow trout muscle tissue. J. Food Sci. 59: 797-799.

24

- 1 Ofstad, R., Kidman, S., Myklebust, R., and Hermansson, A.M. 1993. Liquid holding  
2 capacity and structural changes during heating of fish muscle; cod (*Gadus morhua* L.)  
3 and salmon (*Salmo salar*). *Fd. Struc.* 12: 163-174.
- 4
- 5 Ofstad, R., Kidman, S., Myklebust, R., Olsen, R.L. and Hermansson, A.M. 1995. Liquid  
6 holding capacity and structural changes in comminuted salmon (*Salmo salar*) muscle as  
7 influenced by pH, salt and temperature. *Lebens-Wiss und- Technol.* 28: 329-339.
- 8
- 9 Ofstad, R., Kidman, S., Myklebust, R., Olsen, R.L., and Hermansson, A.M. 1996.  
10 Factors influencing liquid-holding capacity and structural changes during heating of  
11 comminuted cod (*Gadus morhua* L.) muscle. *Lebens-Wiss und- Technol.* 29: 173-183.
- 12
- 13 Olsen, S.H., Sorensen, N.K., Stonno, S.K., and Elvevoll, E.O. 2006. Effect of slaughter  
14 methods on blood spotting and residual blood in fillets of Atlantic salmon (*Salmo*  
15 *salar*). *Aquaculture* 258: 462-469.
- 16
- 17 Rasmussen, R.S. 2001. Quality of farmed salmonids with emphasis on proximate  
18 composition, yield and sensory characteristics. *Aquac. Res.* 32:767-786.
- 19
- 20 Rasmussen, R.S., Rønsholdt, B., Ostefeld, T.H., McLean, E., and Byatt J. C. 2001.  
21 Growth, feed utilisation, carcass composition and sensory characteristics of rainbow  
22 trout treated with recombinant bovine placental lactogen and growth hormone.  
23 *Aquaculture* 195: 367-384.
- 24

1 Refsgaard, H.F.F., Brockhoff, P., and Jensen, B. 1998. Biological variation of lipid  
2 constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon  
3 (*Salmo salar*). J. Agric. Food Chem. 46: 808-812.

4  
5 Regenstein, J.M., Jauregui, C.A., and Baker, R.C. 1984. The effect of pH,  
6 polyphosphates and different salts on water retention properties of ground trout muscle.  
7 J. Food Biochem. 8: 123-131.

8  
9 Regost, C., Arzel, J., Cardinal, M., Laroche, M., and Kaushik, S. J. 2001. Fat deposition  
10 and flesh quality in seawater reared triploid brown trout (*Salmo trutta*) as affected by  
11 dietary fat levels and starvation. Aquaculture 193: 325-345.

12  
13 Regost, C., Jakobsen, J.V. and Rørå, A.M.B. 2004. Flesh quality of raw and smoked  
14 fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. Food  
15 Res. Internat. 37: 259-271.

16  
17 Robb, D.H.F., Kestin, S.C., Warriss, P.D., and Nute, G.R. 2002. Muscle lipid content  
18 determines the eating quality of smoked and cooked Atlantic salmon (*Salmo salar*).  
19 Aquaculture 205: 345-358.

20  
21 Rørå, A.M.B. 2003. Raw material characteristics and treatment – effects on yield and  
22 quality of cold-smoked Atlantic salmon. Ph. D. thesis. Agricultural University of  
23 Norway.

24

- 1 Rørå, A.M.B., and Einen, O. 2003. Effects of freezing on quality of cold-smoked  
2 salmon based on the measurements of physiochemical characteristics. J. Food Sci. 68  
3 (6): 2123-2128.
- 4
- 5 Rørå, A.M.B., Kvåle, A., Mørkøre, T., Rørvik, K-A., Steinen, S.H., and Thomassen,  
6 M.S. 1998. Process yield, colour and sensory quality of smoked Atlantic salmon (*Salmo*  
7 *salar*) in relation to raw material characteristics. Food Res. Internat. 31: 601-609.
- 8
- 9 Rørå, A.M.B., Regost, C., and Lampe, J. 2003. Liquid holding capacity, texture and  
10 fatty acid profile of smoked fillets of Atlantic salmon fed diets containing fish oil or  
11 soybean oil. Food Res. Internat. 36: 231-239.
- 12
- 13 Rørå, A.M.B., Ruyter, B., Skorve, J., Berge, R.K., and Slinning, K-E. 2005a. Influence  
14 of high content of dietary soybean oil on quality of large fresh, smoked and frozen  
15 Atlantic salmon (*Salmo salar*). Aquac. Int. 13: 217-231.
- 16
- 17 Rørå, A.M.B., Birkeland, S., Hultmann, L., Rustad, T., Skåra, T., and Bjerkeng, B.  
18 2005b. Quality Characteristics of farmed Atlantic salmon fed diets high in soybean or  
19 fish oil as affected by cold smoking temperature. J. Food Sci. 38: 201-211.
- 20
- 21 Sargent, J.R., Bell, J.G., McGhee, F., McEvoy, J., and Webster, J. L. 2001. The  
22 nutritional value of fish. In: *Farmed fish quality* (Eds.: Kestin, S. C. and Warris, P.).  
23 Blackwell, Oxford. pp 3-12.
- 24

1 Sato, K., Yoshinak, R., Sato, M., and Shimizu, T. 1986. Collagen content in muscle of  
2 fishes in association with their swimming movement and meat texture. Bull. Jpn. Soc.  
3 Sci. Fish 52 (9): 1595-1600.

4  
5 Sato, K., Ohashi, C., Ohtsuki, K., and Kawabata, M. 1991. Type V collagen in trout  
6 (*Salmo gairdneri*) muscle and its solubility change during chilled storage of muscle. J.  
7 Agric. Food Chem. 39: 1222-1225.

8  
9 Schubring, R. 2006. Thermal stability, texture, liquid holding capacity and colour of  
10 smoked salmon on retail level. Thermochimica Acta. 445: 168-178.

11  
12 Séménou, M., Courcoux, P., Cardinal, M., Nicod. H., and Quisse, A. 2007. Preference  
13 study using a latent class approach. Analysis of European preferences for smoked  
14 salmon. Food Qual. Pref. 18: 720-728.

15  
16 Sheehan, E.M., O'Connor, T.P.O., Sheehy, P.J.A., Buckley, D.J., and FitzGerald, R.  
17 1996. Effect of dietary fat intake on the quality of raw and smoked salmon. Ir. J. Agric.  
18 Food Res. 35:37-45.

19  
20 Shearer, K.D. Åsgård, T., Andorsdottir, G., and Aas, G. H. 1994. Whole body elemental  
21 and proximate composition of Atlantic salmon (*Salmo salar*) during the life cycle. J.  
22 Fish Biol. 44: 785-797.

23  
24 Sigurgisladóttir, S., Torrissen, Lie, Ø. Thomasson, M., and Hafsteinsson, H. 1997.

1 Salmon quality: Methods to determine the quality parameters. Rev. Fish Sci. 5: 223-  
2 252.

3  
4 Sigurgisladóttir, S., Hafsteinsson, H., Jonsson, A., Lie, Ø., Nordvedt, R., Thomasson,  
5 M., and Torrissen, O. 1999. Textural properties of raw salmon fillets as related to  
6 sampling method. J. Food Sci. 64(1): 99-104.

7  
8 Sigurgisladóttir, S., Ingvarsdóttir, H., Torrissen, O. J., Cardinal, M., and Hafsteinsson,  
9 H. 2000a. Effect of freezing/thawing on the microstructure and the texture of smoked  
10 Atlantic salmon (*Salmo salar*). Food Res. Internat. 33 (10): 857-865.

11  
12 Sigurgisladóttir, S., Sigurdardóttir, M. S., Torrissen, O. J., Vallet, J. L., and  
13 Hafsteinsson, H. 2000b. Effects of different salting and smoking processes on the  
14 microstructure, the texture and yield of Atlantic salmon (*Salmo salar*) fillets. Food Res.  
15 Internat. 33: 847-855.

16  
17 Skrede, G., and Storebakken, T. 1986a. Characteristics of color in raw, baked and  
18 smoked wild and pen-reared Atlantic salmon. J. Food Sci. 51 (3): 804-808.

19  
20 Skrede, G. and Storebakken, T. 1986b. Instrumental color analysis of raw, baked and  
21 smoked wild and pen-reared Atlantic salmon. Aquaculture 53 (3-4): 279-286.

22  
23 Storebakken, T., Hung, S.S.O., Calvert, C.C., and Plisetskaya, E.M. 1991. Nutrient  
24 partitioning in rainbow trout at different feeding rates. Aquaculture 96: 191-203.

1

2 Torrissen, O.J. 1995. Hvordan har pigmenteringen av norsk laks utviklet sig? Norsk

3 Fiskeopdrett 18A: 12-13.

4

5 Torrissen, O.J., Sigurgisladottir, S., and Slinde, E. 2001. Texture and technological

6 properties of fish. In: *Farmed Fish Quality* (Eds.: Kestin, S. C and Warris, P.).

7 Blackwell science, Oxford. pp 42-57.

8

9 Torstensen, B.E., Bell, J G., Rosenlund, G., Henderson, J., Graff, I.E., Douglas, R.,

10 Tocher, D., Lie, Ø., and Sargent, J.R. 2005. Tailoring of Atlantic Salmon (*Salmo salar*

11 L.) Flesh Lipid Composition and Sensory Quality by Replacing Fish Oil with a

12 Vegetable Oil Blend. J. Agric. Food Chem. 53(26): 10166 - 10178.

13

14 Veliyulin, E., van der Zwaag, C., Burk, W., and Erikson, U. 2005. *In vivo* determination15 of fat content in Atlantic salmon (*salmo salar*) with a mobile NMR spectrometer. J. Sci.

16 Food Agric. 85:1299-1304

## Figures



Figure 1. Gaping and soft texture



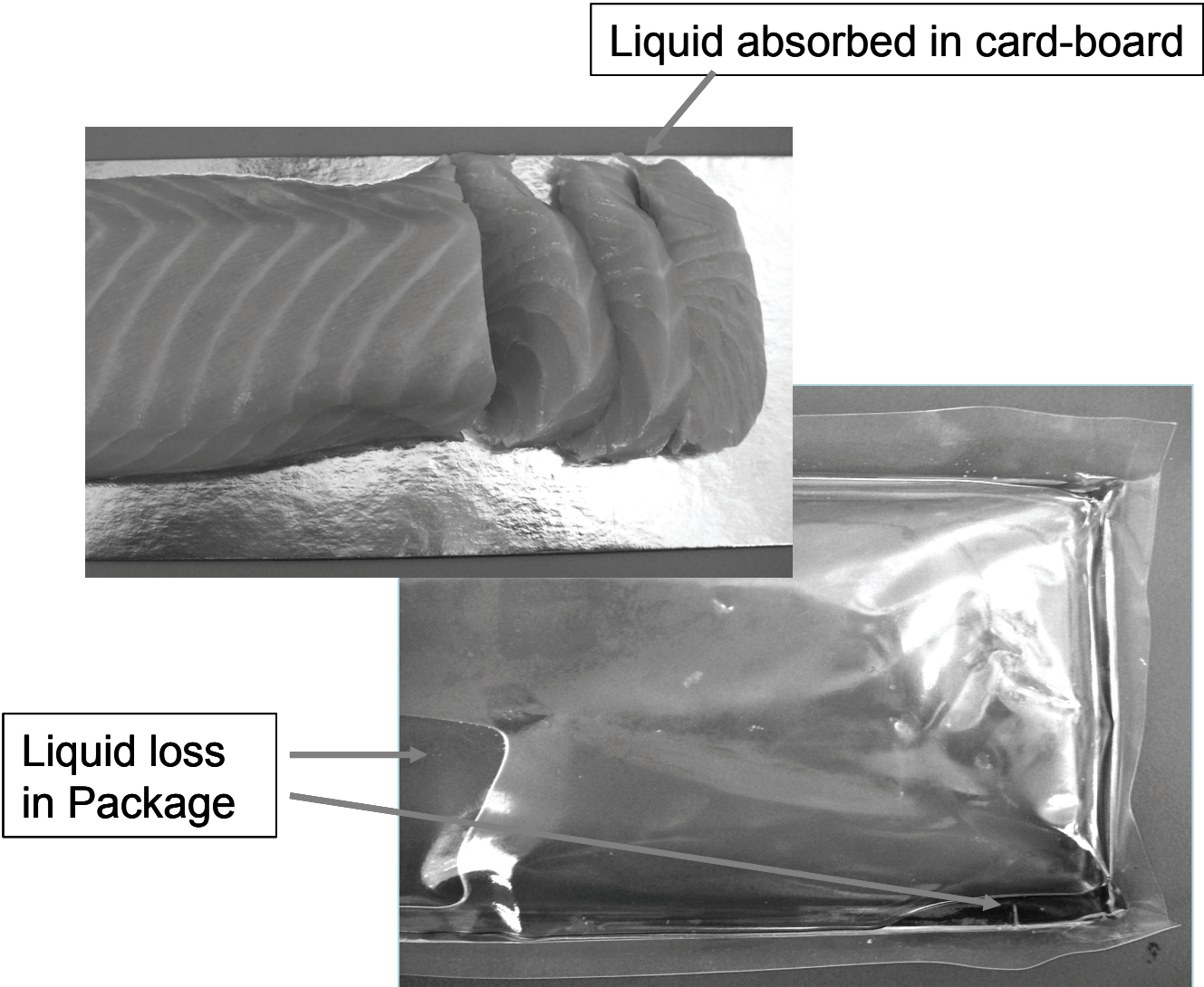
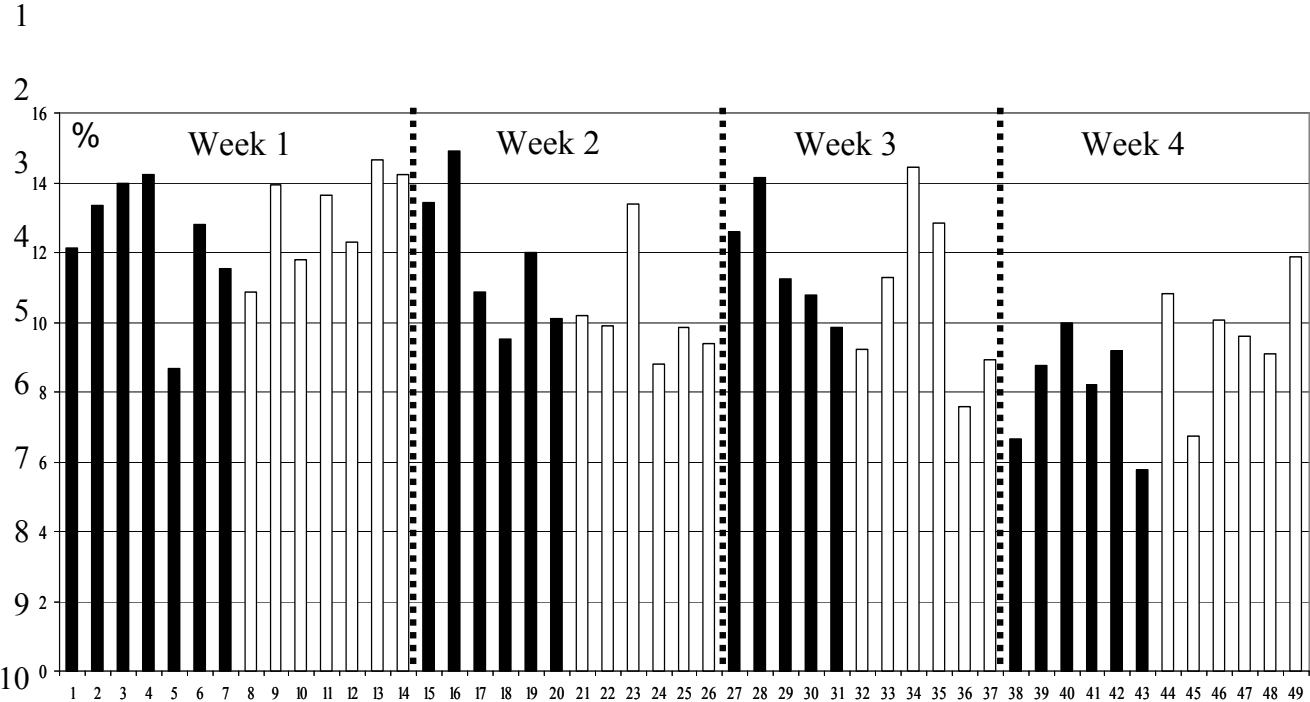


Figure 2. Liquid loss



**Figure 3.** Lipid content (%) in raw salmon. The black columns represent analysis of one box of salmon and white columns represent another box of salmon from the same batch. Thus, four batches were analysed.

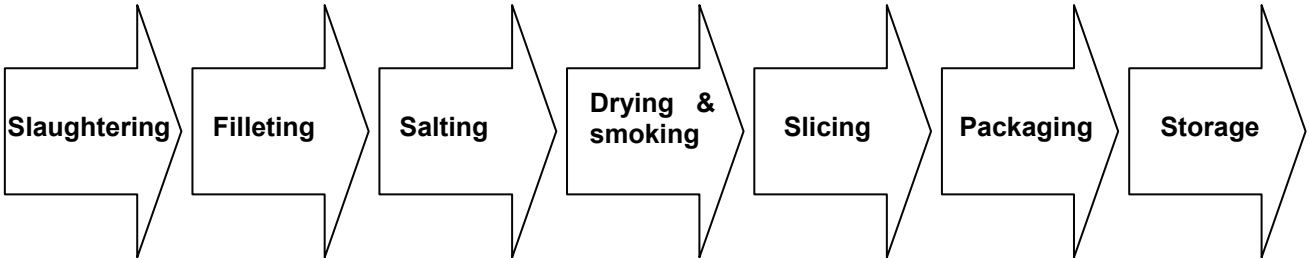


Figure 4. Flow chart showing the process for production of cold smoked salmon.

1

2       **Changes in liquid holding capacity, water distribution**  
3       **and microstructure during chill storage of smoked**  
4       **salmon**

5

6     Hanne Løje, Kristina N Jensen, Grethe Hyldig, Henrik H Nielsen and Jette  
7     Nielsen

8

9     Technical University of Denmark (DTU)

10    Danish Institute for Fisheries Research

11    Department of Seafood Research

12    DTU Søtofts Plads Building 221

13    DK-2800 Kgs. Lyngby

14    E-mail: [hal@difres.dk](mailto:hal@difres.dk)

15

16

17    *Keywords:* Smoked fish, chill storage, microstructure, water distribution, liquid  
18    holding capacity

# 1    **ABSTRACT**

## 2    **BACKGROUND**

3    During recent years, an increase in liquid loss (LL) and soft texture in cold  
 4    smoked salmon has been reported, and these changes may be due to increased  
 5    lipid content and change in muscle structure. These problems can lead to  
 6    downgrading of the products. This study aims to investigate the influence of raw  
 7    material composition and chill storage on quality parameters of cold smoked  
 8    salmon.

9

## 10    **RESULTS**

11    Initial smoked fish had a higher liquid holding capacity (LHC) compared to  
 12    samples stored for 20 days. The large fish lost more liquid than did the small fish.  
 13    The difference in LHC was consistent with a change in water distribution that  
 14    could indicate denaturation of muscle protein. Studies of the microstructure  
 15    showed influence of both the processing and the chill storage. Indication of  
 16    released lipid from the cells was seen after 20 days of chill storage, which could  
 17    be related to a decreased LHC at the same time.

18

## 19    **CONCLUSION**

20    Both the raw material and chill storage affected the quality parameters of smoked  
 21    salmon. This study has improved the knowledge about the relationships between  
 22    muscle structure, liquid-holding properties and water distribution in smoked  
 23    salmon.

## 1 INTRODUCTION

2

3 During recent years, an increase in liquid loss, soft texture, and gaping in cold  
4 smoked salmon products has been reported.<sup>1,2</sup> Such problems with respect to the  
5 product quality can lead to a decrease in retailer and consumer acceptance of cold  
6 smoked products and thus be of economical disadvantage to the producers. It is  
7 therefore important to gain a better understanding of what initiates these  
8 deteriorations in order to maintain a high quality of the products.

9

10 The quality of cold smoked salmon can be influenced by several parameters with  
11 relation to raw material composition,<sup>3,4</sup> processing conditions<sup>5,6,7</sup> and “post-  
12 processing” parameters (shelf life and storage temperature of the product).<sup>8</sup>  
13 Additional parameters such as slaughtering methods,<sup>9,10</sup> genetics and rearing  
14 conditions (density, feed, temperature, stress etc.) can also have an influence on  
15 the product quality.<sup>11</sup>

16

17 Water and lipid together make up about 80 % of the fish muscle. The content of  
18 water and its distribution within the flesh has a significant influence on parameters  
19 such as appearance, liquid holding capacity, texture and storage-stability. Muscle  
20 tissue water can be regarded as being distributed into several domains or “pools”  
21 according to its interaction with and/or entrapment by structural elements of the  
22 cells.<sup>12</sup> Changes in the chemical composition and physical structure influence the  
23 distribution of the water between these pools.<sup>12,13</sup> In addition, storage and

1 processing parameters such as storage time, salting, pH, heating, freezing and  
 2 mincing can have an influence.

3

4 Farmed salmon have a higher lipid content compared to the traditionally used wild  
 5 salmon.<sup>14</sup> A high lipid content has been reported to affect lipid and water loss in  
 6 smoked salmon whether vacuum packed sliced or as whole fillets.<sup>15</sup> It has also  
 7 been reported to affect texture and colour.<sup>16,17</sup> Variation in lipid content leads to  
 8 changes in the water fraction and that may be reflected in the water distribution as  
 9 shown for herring, where water distribution varied according to lipid content and  
 10 the number of water pools was related to the seasonal changes in lipid content.<sup>18</sup>

11

12 Problems with gaping and soft texture in salmon have been linked to the variation  
 13 in collagen content and collagen composition,<sup>3</sup> as well as high lipid content.<sup>17</sup>

14 These textural problems may result in difficulties in slicing the smoked fish.

15 Collagen in raw fish muscle is known to soften after few days of chill storage<sup>19,20</sup>  
 16 and it is also affected by heating as the denaturation and shrinkage temperature of  
 17 fish collagens are close to 20 °C and 40 °C, respectively.<sup>21</sup>

18

19 Little information is available on the effect of the cold smoking process on the  
 20 microstructure of the fish muscle. The smoking process consists of two steps,  
 21 salting and smoking, at temperatures between 20 °C and 30 °C and many  
 22 variations in time and temperature for the process exist among the different smoke  
 23 houses. Ofstad *et al.*<sup>22, 23, 24</sup> have reported on the effects of salt and heating on raw  
 24 salmon, while Sigurgisladottir *et al.*<sup>25,26,27</sup> have investigated the effect of different

1 biological characteristics, different process parameters and effects of  
 2 freezing/thawing on the microstructure of cold smoked salmon. Ofstad *et al.*<sup>22</sup>  
 3 found that the extracellular space became widened with increasing temperature,  
 4 even though no shrinkage of the muscle cell could be seen (upon heating to 20  
 5 °C). In addition a relationship between the water-holding capacity of the fish  
 6 muscle and tissue-specific structural changes were observed during heating. Upon  
 7 salting, the fish muscle lost its structural order<sup>23</sup>. Sigurgisladottir *et al.*<sup>27</sup> found  
 8 shrinkage of the muscle fibres and dispersion of fat globules among the muscle  
 9 fibres after the salting and smoking process.

10

11 Improved knowledge about the relationships between muscle structure, liquid  
 12 holding properties and water distribution is important to understand how the  
 13 different processing parameters influence the quality of the end product. In the  
 14 present study, changes in liquid holding capacity, water distribution and  
 15 microstructure were related to chill storage of smoked salmon for up to 20 days  
 16 after smoking. The changes were also related to pH and contents of lipid and  
 17 collagen in the muscle.. The structural changes in the samples were investigated  
 18 by light microscopy.

19

## 20 MATERIAL AND METHODS

### 21 Experimental design

22 Four groups of Atlantic salmon (*Salmo salar*) were analysed. Two groups of  
 23 salmon, small and large, were taken from two different farms in Norway.



1 Information about the samples is given in Table 1. The smoked samples were  
2 chill-stored for up to 20 days after smoking. Each group was analysed three times  
3 during the storage period.

4

#### 5 **Preparation of samples**

6 The fish were filleted and trimmed by hand. The left fillet was kept as raw  
7 (unprocessed). The piece under the dorsal fin (piece two) was used for  
8 microscopy. The piece corresponding to the Norwegian quality cut (piece three)  
9 was used for determination of lipid and collagen contents and for determination of  
10 pH (Figure 1).

11

12 The right fillets were dry-salted (60 g salt per kg fillet) for five hours for small  
13 fish (size 3-4 kg) and eight hours for the large fish (size 6-7 kg) at room  
14 temperature. Fine salt with a grain size 0.6-0.13 mm (Brøste, Denmark) was used  
15 and spread over the fillet side and the fillets were left on racks. The brine was able  
16 to run off. Afterwards the fillets were rinsed and chilled at 2 °C until the next day.  
17 An automatic oven was used for smoking. The fillets were dried for 2 hours at 26  
18 °C and smoked in an oven supplied with smoke generated from beechwood for  
19 5½ hours at 26 °C with a relative humidity of 75 %. After smoking the fillets  
20 were chilled at 2 °C until next day.

21

22 The smoked fillets were cut into four pieces (Figure 1). Pieces one to three were  
23 each further divided into three smaller pieces. Each piece was vacuum-packed and  
24 randomly assigned to one of the three storage periods (1, 11 or 20 days) at 2 °C

1 after smoking. The temperature of 2 °C was chosen due to practically reason due  
2 to the large number of samples.

3

4 From the smoked fillets, piece one (in front of the dorsal fin and above the lateral  
5 line) was used for determination of liquid holding capacity (centrifugation  
6 method) and determination of dry matter. Piece two under the dorsal fin was used  
7 for microscopy. Piece three corresponding to the Norwegian quality cut was used  
8 for determination of lipid and collagen contents and for determination of pH.

9

10 The skin was removed from all the samples before analysis. On each day of  
11 analysis, determination of liquid holding capacity and dry matter content, together  
12 with collection of samples for microscopy were performed. The pieces for  
13 determination of chemical composition (piece three) were frozen to -40 °C and the  
14 analyses were performed within one month.

15

16

#### 17 *Preparation of samples for microscopy*

18 Blocks were excised from the muscle part below the dorsal fin and one centimetre  
19 above the lateral line, the dry surface (the smoke hue) was removed. The muscle  
20 blocks were cut into small cubes (1 x 1 x 0.5 centimetre) with a razor blade. The  
21 samples were embedded in tissue tex O. C. T. Compound (embedding medium,  
22 Tissue Tex, USA) and frozen in liquid nitrogen. The frozen specimen was stored  
23 at -80 °C until sectioning.

24

1 The specimens were sectioned (10  $\mu$ m) frozen at – 26 to – 28 °C in a freezing  
 2 microtome (2800 Frigocut N) for transverse cuts. The cryosections were mounted  
 3 on poly-L Lysine slides. The sections were stained with Orange G and Methyl  
 4 blue according to Sigurgisladottir *et al.*<sup>27</sup> with glycerol as mounting media.  
 5 Collagen was stained blue and protein yellow.

6

## 7 **Methods**

8

### 9 *Liquid holding capacity*

10 The liquid holding capacity (LHC) was determined on minced fillet from piece  
 11 one using the centrifugation method by Eide *et al.*<sup>28</sup>. The mince was prepared by  
 12 mincing for 2 x 5 sec at 5 °C in a Knifetec, 1095 Sample Mill (Foss Tecator,  
 13 Sweden). The samples (2 g) were weighed, placed in plastic tubes with a special  
 14 filter bottom (pore size 0.1 mm), centrifuged (1500 g, 10 °C, 5 min) and weighed  
 15 again. LHC was calculated in two ways: as the amount of liquid left in the mince  
 16 after centrifugation relative to either the original amount of liquid (LHC<sub>1</sub>) or the  
 17 dry matter content (LHC<sub>2</sub>). The measurement of LHC was performed in  
 18 quadruplicate. Fatty free dry matter content was used in the calculations of LHC.

19

### 20 *Analysis of microstructure*

21 The thin sections, stained for collagen and protein, were examined in a light  
 22 microscope (LM) (Olympus BX51) with a camera (Olympus, Denmark). From  
 23 each of the four groups of salmon, one series (raw, smoked day 1, smoked day 11  
 24 and smoked day 20) were stained and investigated for structural changes using

1 light microscopy. For each sample two to four specimens were examined and  
 2 about 5 to 10 images were obtained for each specimen.

3

#### 4 *Analysis of pH, lipid and collagen content*

5 Analysis of pH, lipid and collagen contents was performed on the area behind the  
 6 dorsal fin (Norwegian quality cut). The piece was minced for 2 x 5 sec at 5 °C in a  
 7 Knifetec, 1095 Sample Mill (Foss Tecator, Sweden). The lipid content was  
 8 determined by a modified version of the Bligh & Dyer extraction method.<sup>29</sup> The  
 9 collagen content was determined according to ISO<sup>30</sup> using sulphuric acid instead  
 10 of perchloric acid to dissolve the colour reagent. Measurement of pH was  
 11 performed with an Autocal pH meter (Metrohm, Denmark). All analyses were  
 12 performed in replicate.

13

#### 14 **Data analysis**

15 The relationships between chemical and functional properties (response variables)  
 16 were explored by Partial Least Square Regression (PLSR) using the  
 17 Unscrambler® version 9.1 (CAMO, Oslo, Norway). PLSR model was calculated  
 18 for intact smoked salmon samples, and the relationship between response variable  
 19 (X) and design variables (Y) was explored. The water pool data used in in PLSR  
 20 model where those reported by Løje *et al.*<sup>31</sup> The Y-matrix consisted of indicator  
 21 variables (values either zero or one) for storage day s (1, 11 or 20 days) and group  
 22 of fish (farm 1 small, farm 2 small, farm 1 large or farm 2 large). All the models  
 23 were calculated with segmented cross-validation with 10 % of the samples,  
 24 randomly selected, in each segment. The single response variables were mean-

1 centred and weighted with  $1/SD$  ( $SD$  being the standard deviation for each  
 2 variable across samples), while 0/1 data was not weighted. An optimal number of  
 3 components were determined as the number of components corresponding to the  
 4 highest explained and validated  $Y$ -variation. Statistical tests included one-way  
 5 analysis of variance (ANOVA),  $t$  test and calculation of Pearson correlation. In  
 6 cases, where sample groups were not normally distributed, a non-parametric  
 7 correlation, Spearman was chosen. Statistical test were performed with GraphPad  
 8 Prism version 4 (GraphPad Software, San Diego, CA, USA).

9

## 10 **RESULTS AND DISCUSSIONS**

### 11 **Variation in chemical and functional parameters due to chill storage**

12 PLSR model was calculated for intact samples (called B in <sup>31</sup>) smoked salmon  
 13 with the  $X$ -matrix consisting of response variables (chemical and functional  
 14 properties) and the  $Y$ -matrix consisting of indicator variables (values either zero  
 15 or one) for the four groups and the three storage times after smoking. The  
 16 correlation loadings for the first two components from the model of intact smoked  
 17 samples are shown in Figure 2. The first component mainly explains the  
 18 difference between the four groups. In particular, a difference between small and  
 19 large fish can be seen and this may be related to differences in e.g. lipid contents.  
 20 The lipid contents are shown for raw sample in Table 2 and for smoked samples  
 21 in Table 3 The large fish (size 6-7 kg) had higher lipid contents ( $P < 0.001$ ) than  
 22 the smaller fish (size 3-4 kg). A high lipid content has been reported to lead to  
 23 decreased liquid holding capacity (LHC). Mørkøre *et al.*<sup>15</sup> found a negative  
 24 correlation between lipid content (between 14.5 and 21.8 %) and LHC

1 (determined by the centrifugation method by Gomez-Guillén *et al.*<sup>32</sup> in fillets of  
 2 smoked salmon. Birkeland *et al.*<sup>6</sup> found a decreased LHC (measured by a higher  
 3 lipid loss) for smoked salmon with high lipid contents ( $20.2 \pm 1.1\%$ ) than for  
 4 smoked salmon with low lipid contents ( $16 \pm 1.0\%$ ). In the present study, a  
 5 significant ( $P < 0.0001$ ) correlation of -0.71 between lipid content and LHC  
 6 according to original liquid content ( $LHC_1$ ) for smoked salmon was found.  $LHC_1$   
 7 is calculated by dividing with the liquid (water and lipid) content. An in-directly  
 8 relation to the lipid content is expected. No correlation between lipid content and  
 9 liquid holding capacity according to dry matter ( $LHC_2$ ) was found for smoked  
 10 samples.

11

12

13 In Figure 2, the second component was related to a development during chill  
 14 storage of the smoked samples and the greatest change was seen between day 11  
 15 and day 20. This change was related to a change in LHC as a decrease was  
 16 observed between day 11 and day 20 ( $P < 0.01$ ) after smoking. Rørå *et al.*<sup>8</sup> found  
 17 that storage time (5 or 15 days) at temperature (4 °C to 14 °C) strongly affected  
 18 LHC in smoked salmon as LHC decreased (seen by increase in liquid losses)  
 19 when the temperature increased and when the storage time increased. The liquid  
 20 losses were more pronounced when the two conditions were combined, where a  
 21 liquid loss of 4.4 % was found after 15 days of storage at 14 °C. In the present  
 22 study, the small smoked samples from both farms had a liquid loss of 5 % after  
 23 one day of storage after smoking and a liquid loss of 7 % after 20 days of storage  
 24 after smoking. The large smoked samples could be separated according to the two

1 farms as the large fish from farm one had a liquid loss of 14 % after one day of  
 2 storage after smoking and a liquid loss of 19 % after 20 days of storage after  
 3 smoking, while large smoked samples from farm two had a liquid loss of 9 %  
 4 after one day of storage after smoking and a liquid loss of 9 % after 20 days of  
 5 storage after smoking. One of the reasons for the difference in liquid loss for large  
 6 fish could be that the fish were from different farms. However other factors may  
 7 also have an influence. The liquid losses found in the present study were higher  
 8 than those found by Rørå *et al.*<sup>8</sup>. In the present study fish of 3-4 kg and 6-7 kg  
 9 were used, while Rørå *et al.*<sup>8</sup> used fish of 1-1.5 kg. Large fish lose more liquid  
 10 and this may explain the differences between the studies. As the size of the fish  
 11 has an influence on the liquid loss, it is difficult to compare the results to see an  
 12 effect of the different storage temperatures.

13  
 14 The observed changes for LHC may be related to changes seen in water  
 15 distribution. The water pool data used in in PLSR model where those reported by  
 16 Løje *et al.*<sup>31</sup> Løje *et al.*<sup>31</sup> found that the water distribution in the smoked salmon  
 17 was affected by the chill storage as a decrease in the size of water pool II and a  
 18 concurrent increase in the size of water pool I was seen during the chill storage  
 19 from day 1 to day 20 after smoking, with the greatest change happening between  
 20 day 1 and day 11. In a study of thawed cod packed in a modified atmosphere, a  
 21 similar change in water distribution was also observed during chill storage, where  
 22 an increase in the size of water pool I at the expense of pool II was found to  
 23 correspond to the denaturation of muscle protein and a simultaneous change in  
 24 pH.<sup>33</sup>

1  
 2 Ofstad *et al.*<sup>23</sup> found that pH greatly influenced the liquid holding properties in  
 3 raw salmon as an increase in pH from 6.0 to 7.0 (model system) increased the  
 4 LHC. In the present study, the pH varied between 6.2 and 6.3 for the raw salmon  
 5 and between 5.9 and 6.0 for the smoked fish. In the present study the LHC  
 6 decreased while the pH did practically not change in contrast to the result of  
 7 Ofstad *et al.*<sup>23</sup>. Though it should be noted that the pH changes in the present study  
 8 was on a much smaller scale than in the study by Ofstad *et al.*<sup>23</sup>.

9  
 10  
 11 The total collagen content varied between 3.8 to 5.2 g kg<sup>-1</sup> for raw salmon and 3.8  
 12 to 6.1 g kg<sup>-1</sup> for smoked salmon. This is in agreement of what Eckhoff *et al.*<sup>34</sup> (6.6  
 13 g kg<sup>-1</sup>) and Espe *et al.*<sup>3</sup> (2.4 to 4.1 g kg<sup>-1</sup>) found in raw farmed Atlantic salmon. In  
 14 Tables 2 and 3 the total collagen content (g kg<sup>-1</sup>) are shown for raw and smoked  
 15 samples. The lipid content was correlated to the total collagen content ( $r = -0.35$ ,  
 16  $p < 0.01$ ) for smoked salmon. Thus, high lipid content was related to low total  
 17 collagen for smoked salmon. To investigate if the relation between lipid content  
 18 and collagen content were a result of variation in the dry matter content, the  
 19 collagen content were calculated by fat free dry matter. No correlation was found  
 20 between the total collagen content per fat free dry matter and lipid content for the  
 21 smoked salmon. The correlation found between the total collagen (g kg<sup>-1</sup>) and the  
 22 lipid content in smoked salmon was probably due to differences in dry matter  
 23 contents. High collagen content has been associated with firmer texture and less  
 24 gaping incidence,<sup>35,36</sup> while high lipid content in farmed salmon has been



1 associated with softness and gaping,<sup>17</sup> which can result in difficulties when  
2 slicing the smoked fish<sup>34</sup> as more lipid/liquid leaks out when collagen is  
3 degraded. This may indicate a relation between low total collagen content and  
4 high lipid content. However no relation between collagen content and lipid  
5 content was found in the present study.

6

### 7 **Variation in microstructure due to chill storage**

8 From each of the four groups of salmon, one series (raw, smoked day 1, smoked  
9 day 11 and smoked day 20) were stained and investigated for structural changes  
10 using a light microscope (LM). Images of cross-sections of whole muscle from a  
11 small salmon (lipid content 11.5 %) and a large salmon (lipid content 17.1 %):  
12 raw, day 1, day 11 and day 20 after smoking are shown in Figures 3 and 4,  
13 respectively. Muscle cells are stained yellow and collagen is stained blue.

14

15 For the smoked samples, the cells after smoking were more tightly bound than in  
16 the raw samples. This was established by counting the number of cells along a  
17 diagonal line, drawn across the image and it showed that the raw samples had  
18 fewer cells compared to the smoked samples. After 20 days of chill storage of the  
19 smoked product, the extracellular space between the cells became wider. Both the  
20 salting step followed by a smoking step that includes heating at 20-25 °C can  
21 affect the properties of muscle proteins and thereby the muscle structure.

22 Sigurgisladottir *et al.*<sup>25,26,27</sup> found that muscle fibres shrank during the salting and  
23 smoking process. This was measured by calculating the number of muscle fibres  
24 as a percentage of the total number of fibres in a defined cross-sectional area.

1

2 For the raw samples (Figure 3a and 4a) some small gaps were seen within the  
3 muscle. This may be caused by damage due to growth of ice-crystals during  
4 freezing in the preparation step. For the smoked samples, this was not observed  
5 and it may be due the fact that cells are more tightly bound after the smoking  
6 process.

7

8 Sigurgisladdottir *et al.*<sup>27</sup> found that lipids in smoked salmon muscle were released  
9 from the fat cells and floated freely as fat droplets between the muscle fibres. In  
10 the present study, there appears to be larger spots in the extracellular space after  
11 20 days of chill storage, which could be an indication of more released lipid from  
12 the cells into the extracellular space.

13

14 The denaturation and shrinkage temperatures of collagen are near 20 and 40 °C,  
15 respectively.<sup>21</sup> Liquid loss between 20 and 35 °C could be due to the denaturation  
16 of collagen which alters the physical properties of pericellular layer which  
17 represents a physical barrier to the release of fluid.<sup>37</sup> In the present study a  
18 decreased LHC was observed during the chill storage of smoked salmon. It might  
19 be possible that changes in collagen during the cold smoking process affect the  
20 LHC in the muscle due to an alteration of the physical properties of the  
21 pericellular layer.<sup>23,38</sup>

22

23 Images from Confocal laser scanning microscopy (CLSM) of the same samples  
24 (specimen with a thickness of 20 µm and stained with Nile blue) indicated the

1 same pattern in the changes during chill storage of the smoked salmon as  
2 observed with the LM images (results not shown). Fat globules were dispersed  
3 among the muscle fibres after the salting and smoking process. This was also  
4 observed by Sigurgisladottir *et al.*<sup>27</sup>.

5

## 6 CONCLUSION

7 This study has shown that knowledge about the relationships between muscle  
8 structure, liquid holding capacity and water distribution can be used to understand  
9 what happens during processing and during chill storage and with this the  
10 knowledge, the quality of the end product can be enhanced. The results have  
11 shown that the smoked salmon products were affected by the chill storage after  
12 smoking. LHC decreased during chill storage and the large fish lost more liquid  
13 during storage than small fish. Salting and smoking as well as chill storage  
14 affected the microstructure of the smoked products. Indication of released lipid  
15 from the cells was seen after 20 days of chill storage, which could be related to an  
16 increased liquid loss at the same time.

17

## 18 Acknowledgements

19 The study was financially supported by the Danish Ministry of Food, Agriculture  
20 and Fisheries. The authors are very thankful to Ms. Sally Clink for valuable  
21 discussion during preparation of the manuscript, Ms Susan S. Jensen, Mrs. Inge  
22 Holmberg, Mr. Ulrik Cold, Mr. Carsten Østerberg and Mrs. Karin Reimers for  
23 conducting the analytical work.

## REFERENCES

- 1
- 2 1 Espe M, Nortvedt R, Lie Ø and Hafsteinsson H, Atlantic salmon (*Salmo salar*)
- 3 as raw material for the smoking industry. II: Effects of different smoking methods
- 4 on losses of nutrients and on the oxidation of lipids. *Food Chem* **77**: 41-46 (2002).
- 5
- 6 2 Rørå AMB and Einen O Effects of freezing on quality of cold-smoked salmon
- 7 based on the measurements of physiochemical characteristics. *J Food Sci* **68**:
- 8 2123-2128 (2003).
- 9
- 10 3 Espe M, Ruohonen K, Bjørnevik M, Frøyland L, Nortvedt R, and Kiessling A,
- 11 Interactions between ice storage time, collagen composition, gaping and textural
- 12 properties in farmed salmon muscle harvested at different times of the year.
- 13 *Aquaculture* **240**: 489-504 (2004).
- 14
- 15 4 Rørå AMB, Kvåle A, Mørkøre T, Rørvik K-A, Steien SH and Thomassen MS,
- 16 Process yield, colour and sensory quality of smoked Atlantic salmon (*Salmo*
- 17 *salar*) in relation to raw material characteristics. *Food Res Int* **31(8)**: 601-609
- 18 (1998).
- 19
- 20 5 Birkeland S, Skåra T, Bjerkeng B and Rørå AMB, Product yield and gaping in
- 21 cold-smoked Atlantic salmon (*Salmo salar*) fillets as influenced by different
- 22 injection-salting techniques. *J Food Sci* **68(5)**: 1743-1748 (2003).
- 23

- 1 6 Birkeland S, Rørå AMB, Skåra T and Bjerkeng B, Effects of cold smoking  
2 procedures and raw material characteristics on product yield and quality  
3 parameters of cold smoked Atlantic salmon (*Salmo salar* L.) fillets. *Food Res Int*  
4 **37**: 273-286 (2004).  
5
- 6 7 Birkeland S and Bjerkeng B, The quality of cold-smoked Atlantic salmon  
7 (*Salmo salar*) as affected by salting method, time and temperature. *Int J Food Sci.*  
8 *Technol* **40**: 963-976 (2005).  
9
- 10 8 Rørå AMB, Regost C and Lampe J, Liquid holding capacity. texture and fatty  
11 acid profile of smoked fillets of Atlantic salmon fed diets containing fish oil or  
12 soybean oil. *Food Res Int* **36**: 231-239 (2003).  
13
- 14 9 Erikson U, Sigholt T, and Seland A, Handling stress and water quality during  
15 live transportation and slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture*  
16 **149**: 243–252 (1997).  
17
- 18 10 Olsen SH, Sorensen NK, Stonno SK, and Elvevoll EO, Effect of slaughter  
19 methods on blood spotting and residual blood in fillets of Atlantic salmon (*Salmo*  
20 *salar*). *Aquaculture* **258**: 462-469 (2006).  
21
- 22 11 Rasmussen RS, Quality of farmed salmonids with emphasis on proximate  
23 composition, yield and sensory characteristics. *Aquac Res* **32**: 767-786 (2001).  
24

- 1 12 Hills B, *Magnetic Resonance Imaging in Food Science*. Wiley & Sons, New  
2 York (1998).  
3
- 4 13 Ruan RR and Chen PL, *Water in Foods and Biological Materials. A Nuclear*  
5 *Magnetic Approach*. Technomic Publishing Company, Lancaster (1998).  
6
- 7 14 Haard NF, Control of chemical composition and food quality attributes of  
8 cultured fish. *Food Res Int* **25**: 289-307 (1992).  
9
- 10 15 Mørkøre T, Vallet JL, Cardinal M, Gómez-Guillén MC, Montero P, Torrissen,  
11 OJ, Nordtvedt R, Sigurgisladottir S and Thomassen MS, Fat content and fillet  
12 shape of Atlantic salmon: Relevance for processing yield and quality of raw and  
13 smoked products. *J Food Sci* **66**: 1348-1354 (2001).  
14
- 15 16 Einen O, Mørkøre T, Rørå AMB and Thomassen MS, Feed ration prior to  
16 slaughter - a potential tool for managing product quality of Atlantic salmon  
17 (*Salmo salar*). *Aquaculture* **178**, 149-169 (1999).  
18
- 19 17 Sheehan EM, O'Connor TPO, Sheehy PJA, Buckley DJ and FitzGerald R,  
20 Effect of dietary fat intake on the quality of raw and smoked salmon. *Ir J Agric*  
21 *Food Res* **35**: 37-42 (1996).  
22

- 1 18 Jensen KN, Jørgensen BM, Nielsen J and Nielsen HH, Water distribution and  
2 mobility in herring muscle in relation to lipid content, season, fishing ground and  
3 biological parameters. *J Sci Food Agric* **85**: 1259-1267 (2005).  
4
- 5 19 Sato K, Ohashi C, Ohtsuki K and Kawabata M, Type V collagen in trout  
6 (*Salmo gairdneri*) muscle and its solubility change during chilled storage of  
7 muscle. *J Sci Food Agric* **39**: 1222-1225 (1991).  
8
- 9 20 Montero P and Borderias AJ, Influence of age on muscle connective tissue in  
10 trout (*Salmo irideus*). *J Sci Food Agric* **51**: 261-265 (1990).  
11
- 12 21 Sikorski ZE, Scott DN and Buisson DH, The role of collagen in the quality and  
13 processing of fish. *Crit Rev Food Sci Nutr* **20**: 301-343 (1984).  
14
- 15 22 Ofstad R, Kidman S, Myklebust R and Hermansson AM, Liquid holding  
16 capacity and structural changes during heating of fish muscle; cod (*Gadus morhua*  
17 L. ) and salmon (*Salmo salar* L.). *Fd Struc* **12**: 163-174 (1993).  
18
- 19 23 Ofstad R, Kidman S, Myklebust R, Olsen RL and Hermansson AM, Liquid  
20 holding capacity and structural changes in comminuted salmon (*Salmo salar*)  
21 muscle as influenced by pH, salt and temperature. *Lebens Wiss Techno* **28**: 329-  
22 339 (1995).  
23

1 24 Ofstad R, Egelanddal B, Kidman S, Myklebust R, Olsen RL and Hermansson  
 2 AM, Liquid loss as effected by post-mortem ultrastructural changes in fish  
 3 muscle; cod (*Gadus morhua* L.) and salmon (*Salmo salar*). *J Sci Food Agric*  
 4 **71(3)**: 301-312 (1996).

5

6 25 Sigurgisladottir S, Sigurdardottir MS, Torrissen OJ, Vallet JL and Hafsteinsson  
 7 H, Effects of different salting and smoking processes on the microstructure, the  
 8 texture and yield of Atlantic salmon (*Salmo salar*) fillets. *Food Res Int* **33**: 847-  
 9 855 (2000a).

10

11 26 Sigurgisladottir S, Ingvarsdottir H, Torrissen OJ, Cardinal M and Hafsteinsson  
 12 H, Effect of freezing/thawing on the microstructure and the texture of smoked  
 13 Atlantic salmon (*Salmo salar*). *Food Res Int* **33**: 857-865 (2000b).

14

15 27 Sigurgisladottir S, Sigurdardottir MS, Ingvarsdottir H, Torrissen OJ and  
 16 Hafsteinsson H, Microstructure and texture of fresh and smoked Atlantic salmon,  
 17 *Salmo salar* L., fillets from fish reared and slaughtered under different conditions.  
 18 *Aquac. Res*, **32**: 1-10 (2001).

19

20

21 28 Eide O, Børresen T and Strøm O, Minced fish production from capelin  
 22 (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from  
 23 fatty species. *J Food Sci* **47**: 347-349, 354 (1982).

24



- 1 29 Bligh EG and Dyer WJ, A rapid method of total lipid extraction and  
2 purification. *Can J Biochem Physiol* **37**: 911-917 (1959).  
3
- 4 30 ISO 3496, SA, Meat and Meat products – determination of L (-)  
5 Hydroxyproline content, 2<sup>nd</sup> edition International Organisation for  
6 Standardisation, Geneva, (1994).  
7
- 8 31 Løje H, Green-Petersen D, Nielsen J, Jørgensen BM and Jensen KN, Water  
9 distribution in smoked salmon. *J Sci Food Agric* **87(2)**: 212-217 (2007).  
10
- 11 32 Gómez-Guillén MC, Montero P, Hurtado O and Borderías AJ, Biological  
12 characteristics affect the quality of farmed Atlantic salmon and smoked muscle. *J*  
13 *Food Sci* **65**: 53-60 (2000).  
14
- 15 33 Jensen KN and Jørgensen BM, Effect of storage conditions on differential  
16 scanning calorimetry profiles from thawed cod muscle. *Lebens-Wiss under*  
17 *Technol* **36**: 807-81 (2003).  
18
- 19 34 Eckhoff KM, Aidos I, Hemre G-I and Lie Ø, Collagen content in farmed  
20 Atlantic salmon (*Salmo salar*, L.) and subsequent changes in solubility during  
21 storage on ice. *Food Chem* **62(2)**: 197-200 (1998).  
22

- 1 35 Sato K, Yoshinak R, Sato M and Shimizu T, Collagen content in muscle of  
2 fishes in association with their swimming movement and meat texture. *Bull Jpn*  
3 *Soc Sci Fish* **52(9)**: 1595-1600 (1986).  
4
- 5 36 Hatae K, Tobimatsu A, Takeyama M, and Matsumoto JJ, Contribution of the  
6 connective tissues on the texture difference of various fish species. *Bull Jpn Soc*  
7 *Sci Fish* **52(11)**: 2001-2007 (1986).  
8
- 9 37 Ofstad R. Microstructure and liquid holding capacity in cod (*Gadus morhua*  
10 L.) and salmon (*Salmo salar*) muscle; effects of heating. Dr. Scient. Thesis,  
11 Insititute of Medical Biology, University of Tromsø (1995).  
12
- 13 38 Elvevoll EO, Sørensen NK, Østerud B, Ofstad R and Martinez I, Processing of  
14 marine foods. *Meat Sci* **43**: 265-275. (1996).  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24

Figure 1. Sampling of raw fillet and smoked fillet. Piece one (only for smoked samples) in front of the dorsal fin was used for determination of (NMR) LHC and dry matter (DM). The piece under dorsal fin (piece two) was used for microscopy (one centimetre above the lateral line). Piece three was used for determination of lipid and collagen contents and for determination of pH. For the smoked fillet, piece one to three were each further divided into three smaller pieces. Each piece was vacuum-packed and randomly assigned to one of three storage periods (1, 11 or 20) days at 2 °C after smoking.

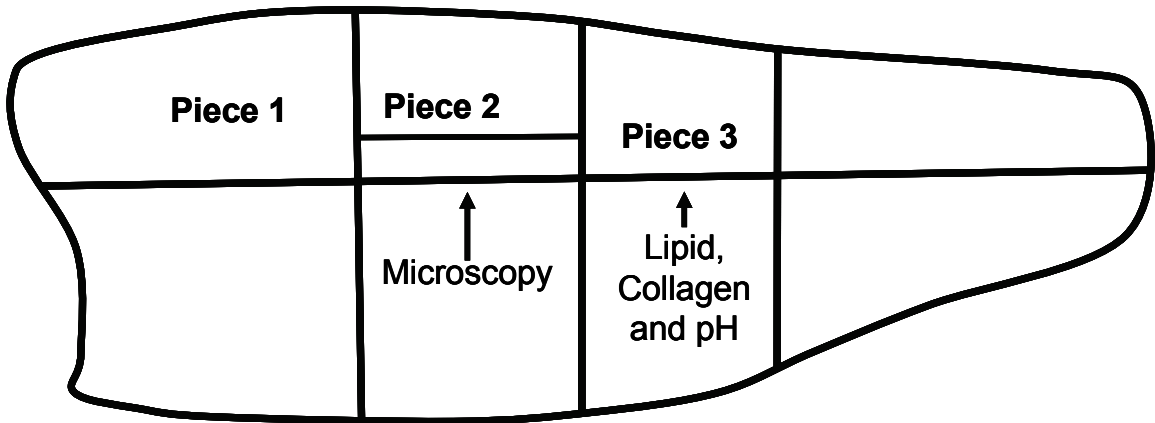
Figure 2. Correlation loadings with factor 2 versus factor 1 from a PLSR model relating the response variable  $X(\bullet)$  and design  $Y(\blacktriangle)$  for intact smoked salmon samples. Day 1, 11 and 20 are days of storage after smoking.  $M_I$ ,  $M_{II}$  and  $M_{III}$  are the relative size of water pool I, II and III, respectively. Collagen is total collagen ( $\text{g kg}^{-1}$ ) LHC<sub>1</sub> the amount of liquid left in the mince after centrifugation relative to either the original amount of liquid. LHC<sub>2</sub> is the amount of liquid left in the mince after centrifugation relative to the dry matter content.

Figure 3. Images obtained by light microscope of cross-sectioned whole muscle from a small salmon: raw (a), day 1 (b), 11 (c) and 20 (d) after smoking. Sections are stained for collagen (blue) and protein (yellow).

Figure 4. Images obtained by light microscope of cross-sectioned whole muscle from a large salmon: raw (a), day 1 (b), 11 (c) and 20 (d) after smoking. Sections are stained for collagen (blue) and protein (yellow).

Figure 1

Raw fillet



Smoked fillet

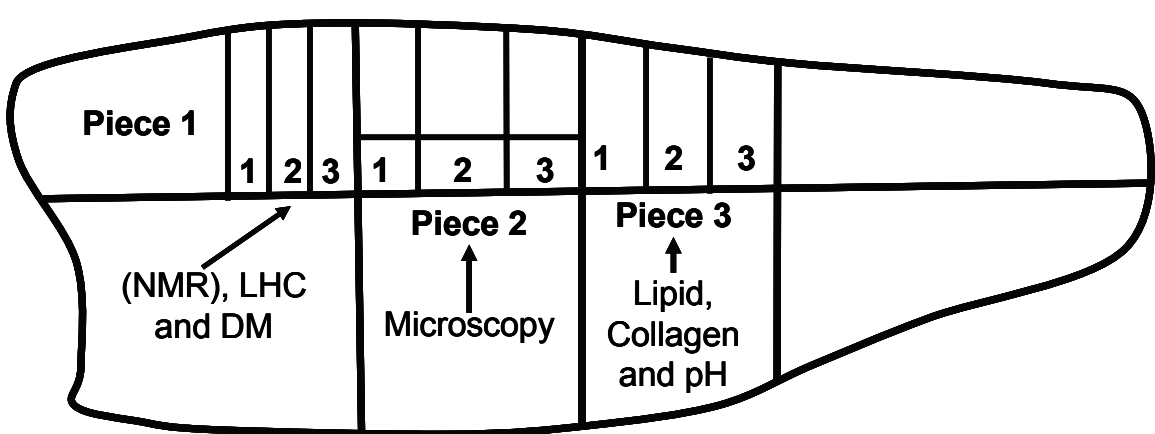


Figure 2

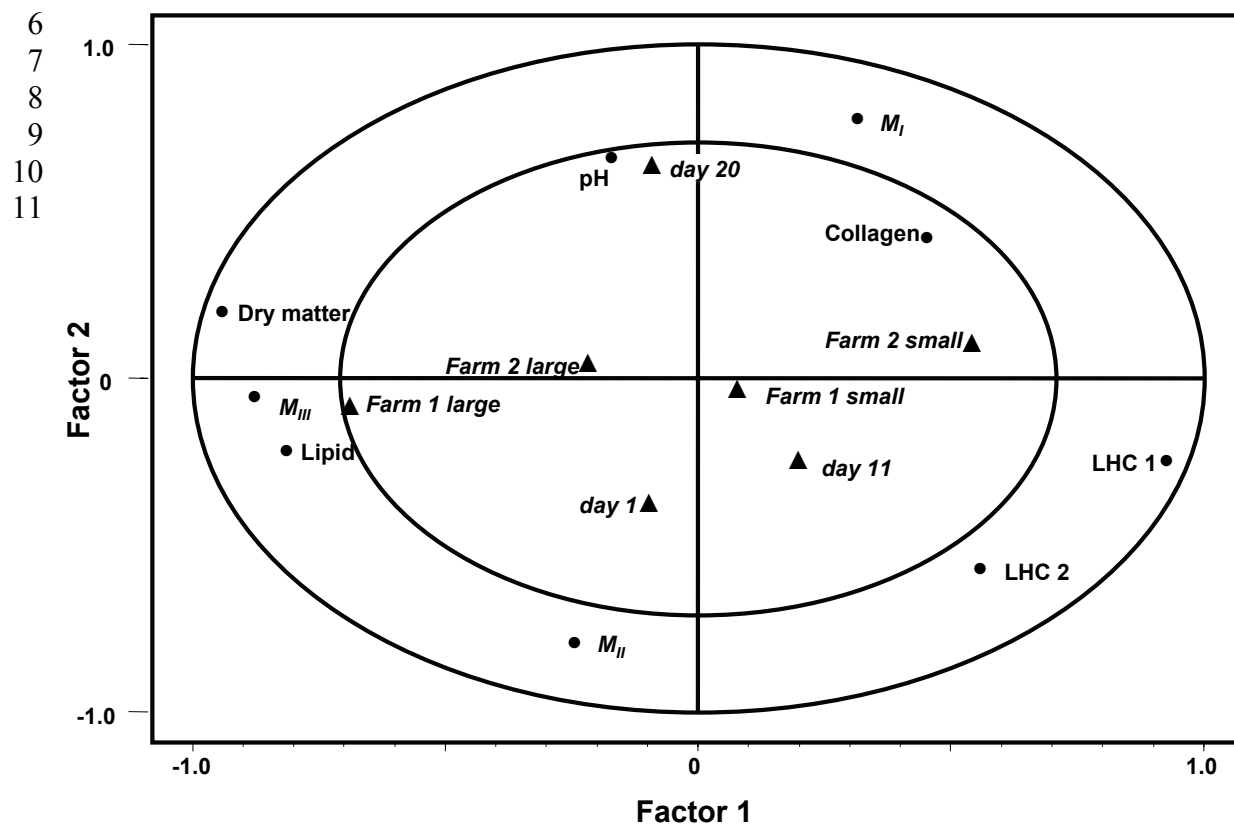


Table 1. Overview of samples.

Group	Description	No. fish	Description	Analysis day
1	Farm 1	7	Small	1, 11 and 20 days after smoking
2	Farm 2	6	Small	1, 11 and 20 days after smoking
3	Farm 1	2	Large	1, 11 and 20 days after smoking
4	Farm 2	3	Large	1, 11 and 20 days after smoking

Small = 3-4 kg, large = 6-7 kg.

Table 2. Lipid content (%), total collagen content (g kg<sup>-1</sup>) and pH values of raw salmon

	Farm 1 small (N = 7)	Farm 2 small (N = 6)	Farm 1 large (N = 2)	Farm 2 large (N = 3)
Lipid content	13.3 ± 0.6	12.5 ± 1.3	18.4 ± 1.9	14.9 ± 1.3
Total collagen	4.3 ± 0.3	4.6 ± 0.4	4.3 ± 0.8	4.7 ± 0.4
pH	6.2 ± 0.03	6.3 ± 0.02	6.3 ± 0.02	6.2 ± 0.01

Values are means ± standard deviation

N is number of samples

Table 3. Liquid holding capacity (LHC<sub>1</sub> and LHC<sub>2</sub>), lipid content (%), total collagen content (g kg<sup>-1</sup>) and pH values of smoked salmon as affected by storage time

Group	Farm 1 small (N = 7)			Farm 2 small (N = 6)			Farm 1 large (N = 2)			Farm 2 large (N = 3)		
	1 day	11 days	20 days	1 day	11 days	20 days	1 day	11 days	20 days	1 day	11 days	20 days
LHC <sub>1</sub>	94.0±2.6	94.6±2.6	92.7±3.7	95.9±2.7	96.8±3.0	93.7±4.8	85.6±1.9	82.7±0.4	80.8±0.1	89.0±3.8	91.9±2.2	89.9±4.3
LHC <sub>2</sub>	2.4 ± 0.2	2.5 ± 0.2	2.2 ± 0.2	2.5 ± 0.2	2.6 ± 0.1	2.3 ± 0.4	2.5 ± 0.5	2.4 ± 0.4	2.0 ± 0.2	2.1 ± 0.2	2.4 ± 0.2	2.3 ± 0.1
Lipid content	12.5 ± 1.3	12.0±1.1	11.4±1.3	10.9±1.2	10.4±1.7	10.2±1.0	17.2±3.4	17.6±2.2	14.9±3.1	12.3±0.3	12.8±0.8	13.8±1.6
Total collagen	4.9 ± 0.4	5.1 ± 0.4	5.3 ± 0.5	5.0 ± 0.3	5.2 ± 0.6	5.3 ± 0.1	4.3 ± 0.6	4.2 ± 0.5	4.7 ± 0.5	5.1 ± 0.3	4.9 ± 0.3	5.0 ± 0.4
pH	6.0 ± 0.04	5.9 ± 0.1	6.0 ± 0.04	6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.02	5.9 ± 0.1	6.0 ± 0.01	6.0 ± 0.01

Values are means ± standard deviation

N is number of samples

LHC<sub>1</sub> = the amount of liquid left in the mince after centrifugation relative to the original amount of liquid

LHC<sub>2</sub> = the amount of liquid left in the mince after centrifugation relative to the dry matter content



Figure 3.

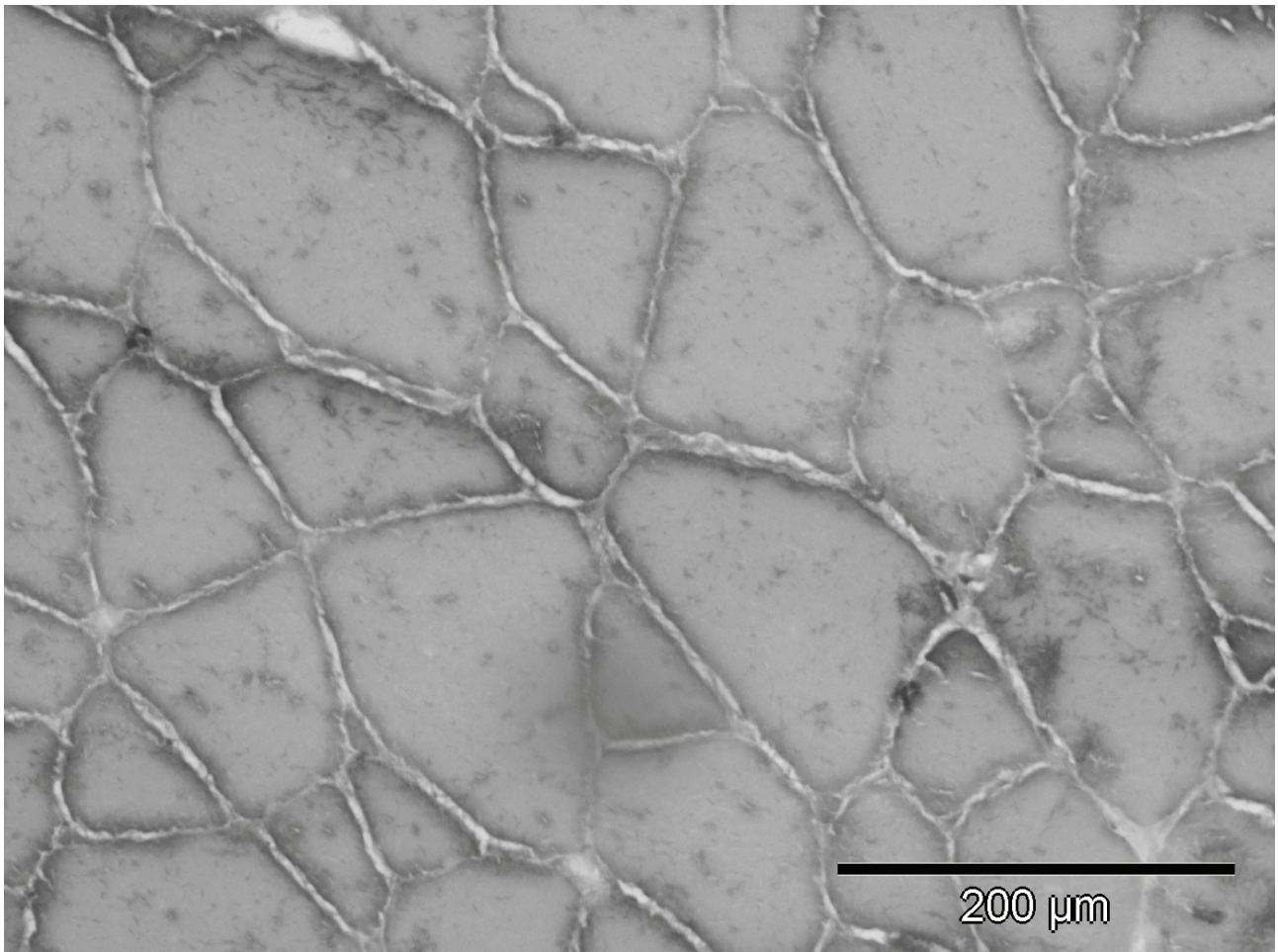


Figure 3a. Image obtained by light microscope of cross-sectioned muscle from a small salmon; raw sample

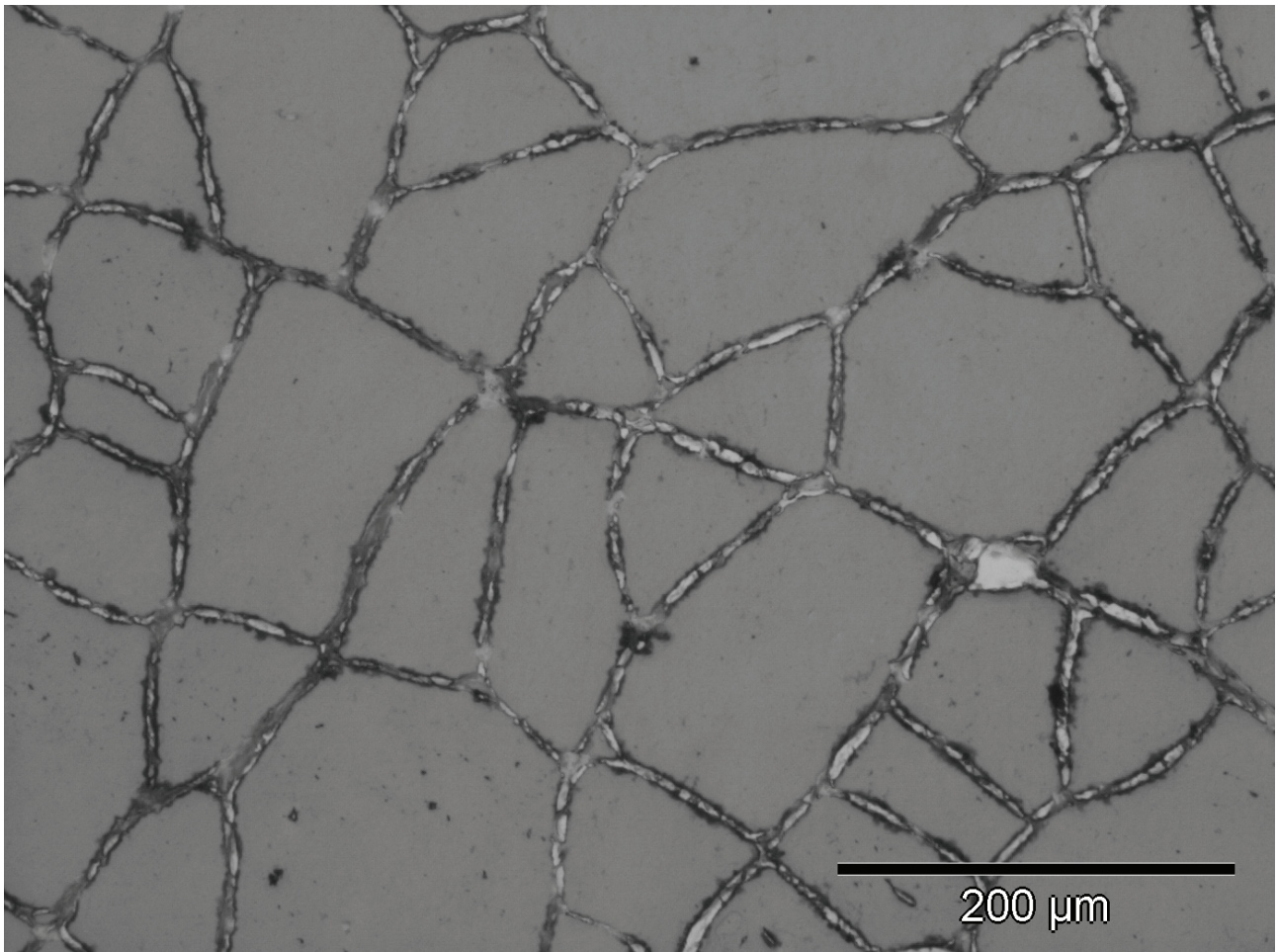


Figure 3b. Image obtained by light microscope of cross-sectioned muscle from a small salmon; smoked sample after 1 day of chill storage after smoking



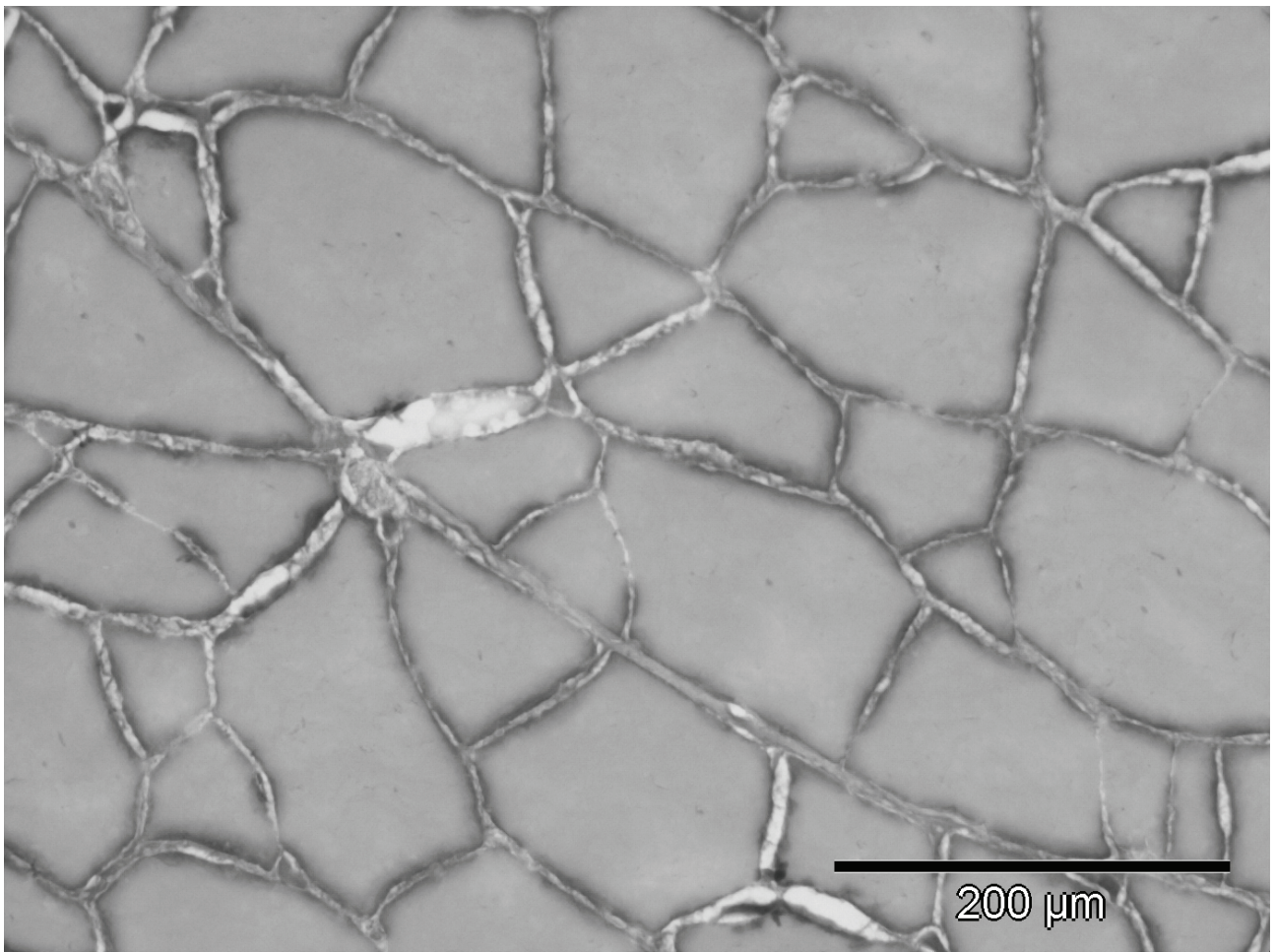


Figure 3c. Image obtained by light microscope of cross-sectioned muscle from a small salmon; smoked sample after 11 days of chill storage after smoking

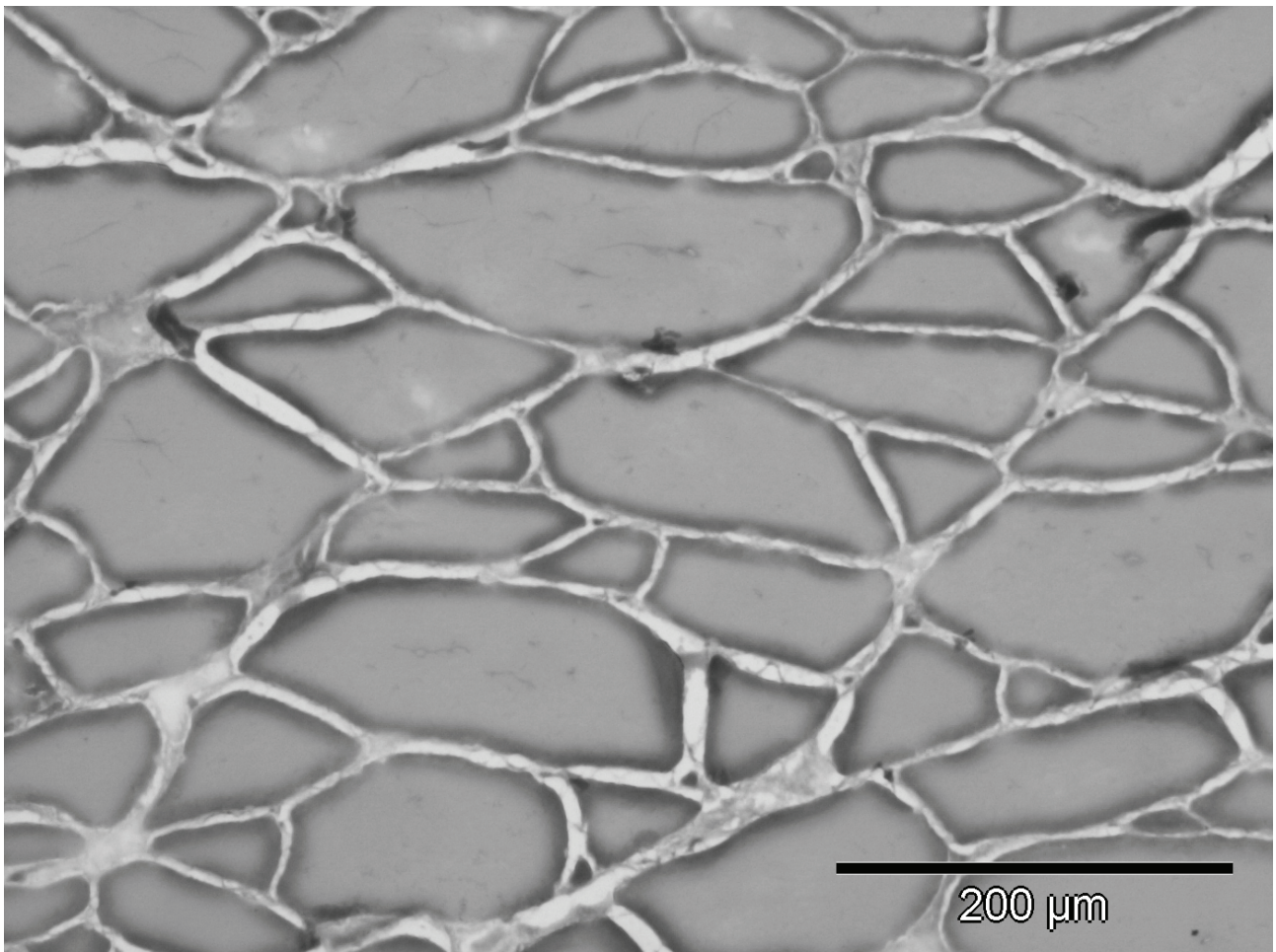


Figure 3d. Image obtained by light microscope of cross-sectioned muscle from a small salmon; smoked salmon after 20 days of chill storage after smoking.



Figure 4.

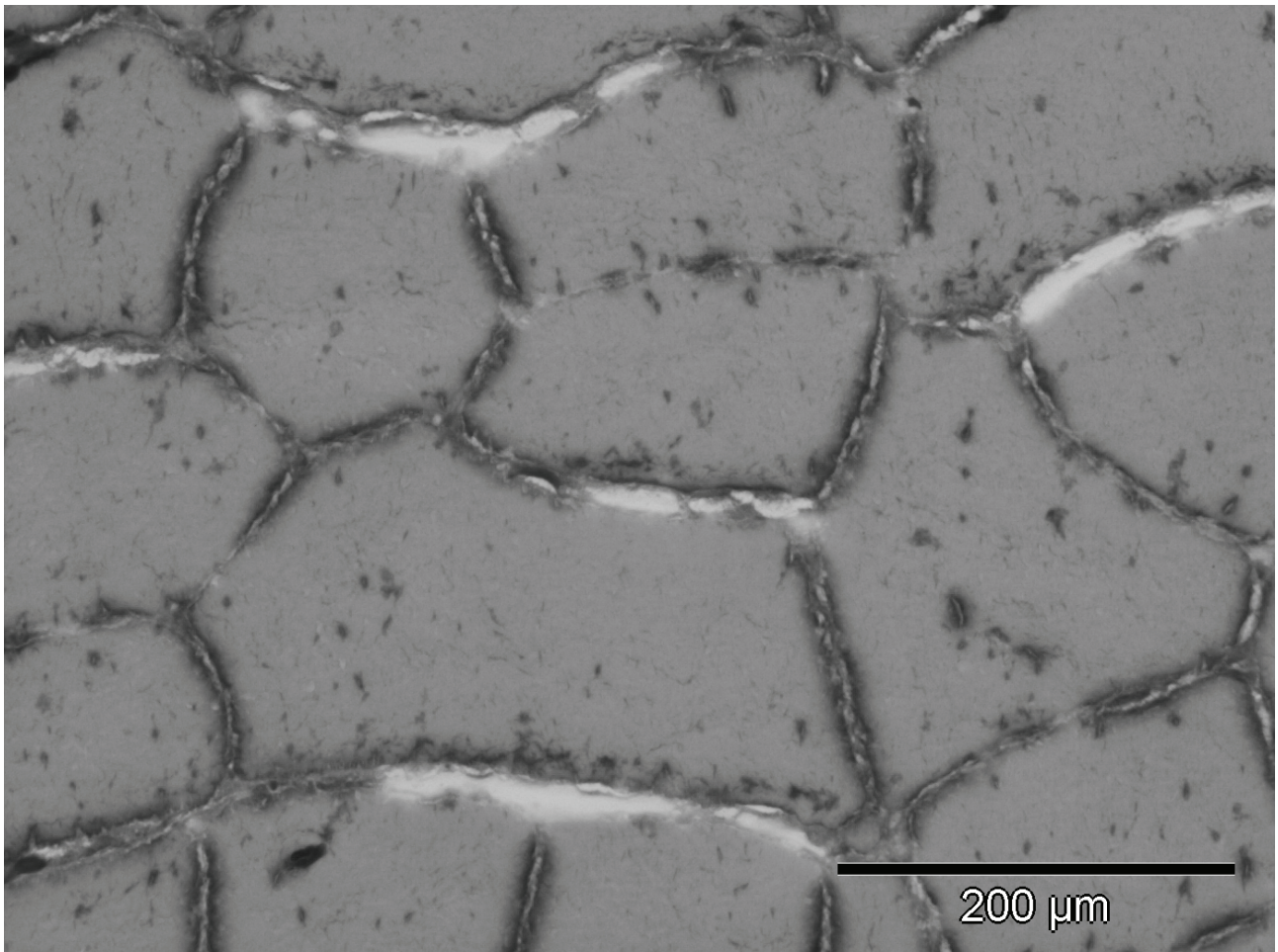


Figure 4a. Image obtained by light microscope of cross-sectioned muscle from a large salmon; raw sample

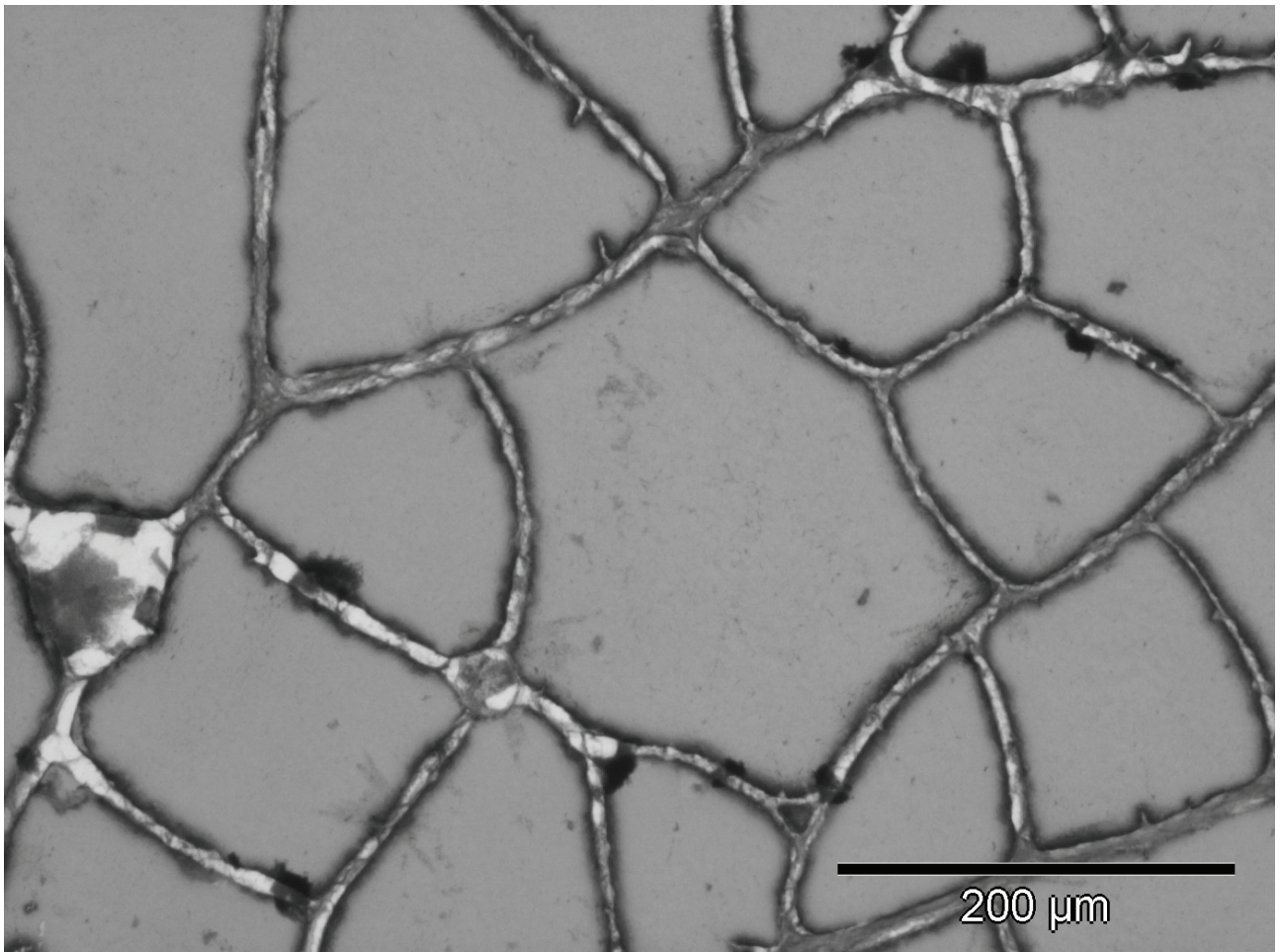


Figure 4b. Image obtained by light microscope of cross-sectioned muscle from a large salmon; smoked sample after 1 day of chill storage after smoking



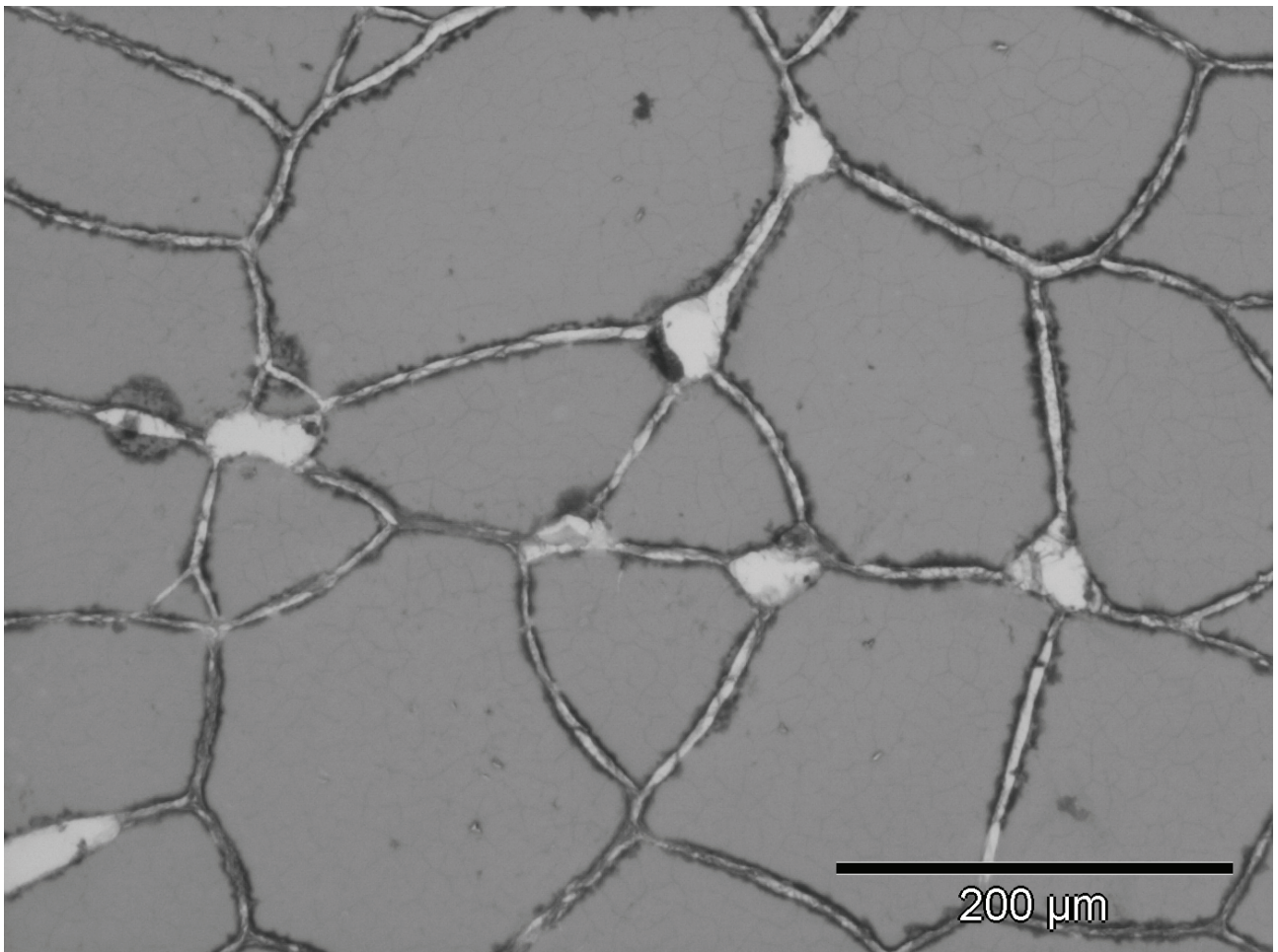


Figure 4c. Image obtained by light microscope of cross-sectioned muscle from a large salmon; smoked sample after 11 day of chill storage after smoking

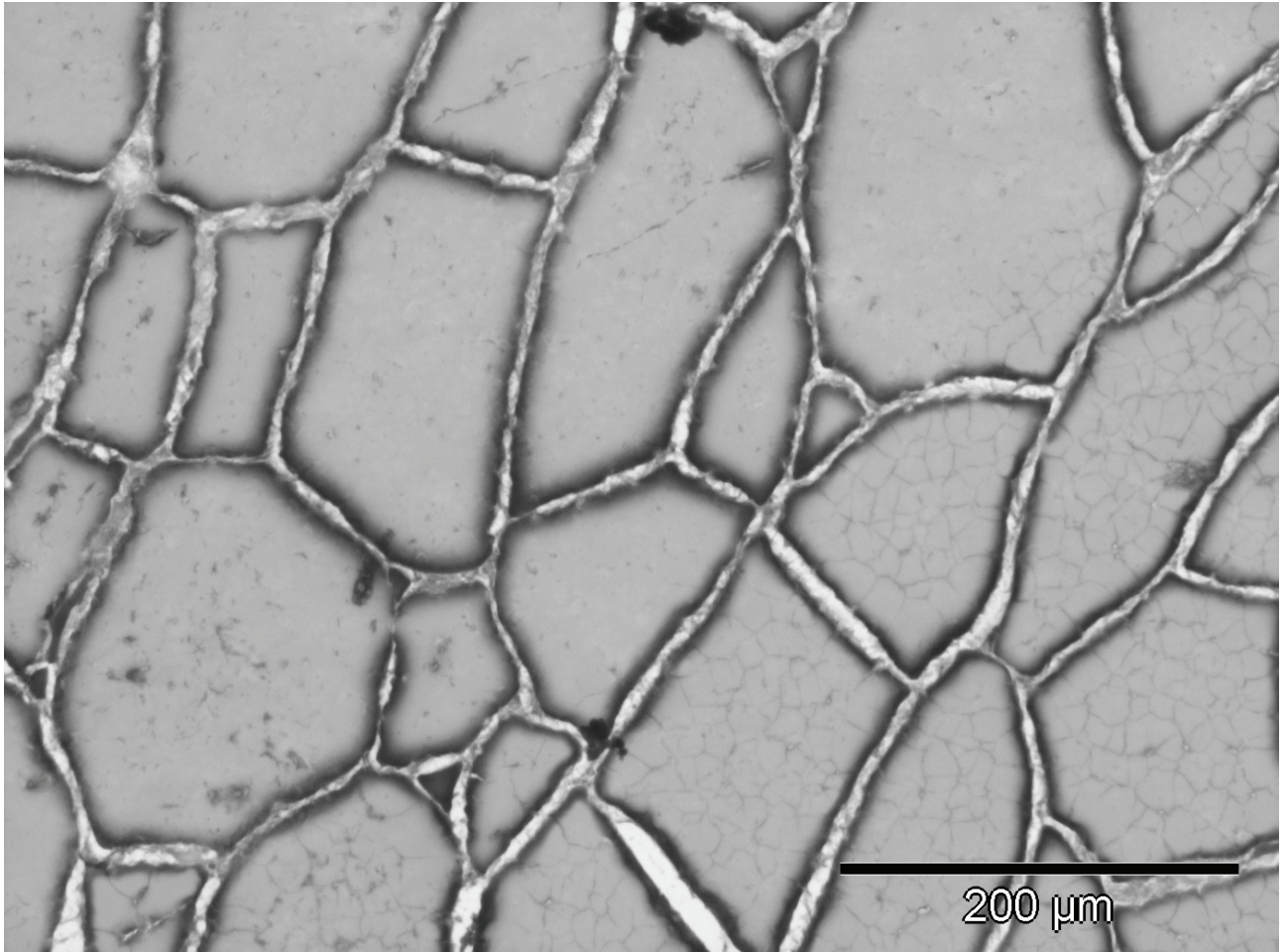


Figure 4d. Image obtained by light microscope of cross-sectioned muscle from a large salmon; smoked sample after 20 day of chill storage after smoking



**Comparison of methods to determine liquid holding capacity in raw and  
smoked salmon and trout**

Hanne Løje\*, Grethe Hyldig, Henrik H. Nielsen, Bo M. Jørgensen & Jette Nielsen

Technical University of Denmark

Danish Institute for Fisheries Research

Department of Seafood Research

Søltofts Plads, Building 221

DK-2800 Kgs. Lyngby, Denmark

\*Corresponding author: Tel.: +45 45 25 49 24, fax: + 45 45 88 47 74

E-mail address: hal@difres.dk

## ABSTRACT

The ability to hold liquid is an important property of cold smoked fish as an increased liquid leakage results in a poorer quality of the product. A suitable measurement of this property, therefore, is advantageous as part of the production control. In the present study, a centrifugation method and a liquid leakage test and low-field NMR were used to determine liquid holding abilities of raw and smoked salmon and rainbow trout. The property 'liquid holding capacity' (LHC), measured by the centrifugation method, was higher for raw fish than for smoked fish. During chill storage at 2°C, a decrease in LHC was observed for both smoked salmon and smoked trout. The property 'liquid loss' (LL), measured by the liquid leakage test, increased during chill storage at 2°C of smoked salmon. For trout, no change in LL was observed during chill storage of the smoked product. The centrifugation method and the liquid leakage test measure different properties. Accordingly, LHC and LL were poorly correlated for smoked salmon as well as for smoked trout. LHC could be determined from low-field NMR relaxation measurements on minced salmon and trout samples. With intact samples, a good relation between NMR relaxation data and LHC was also found. Determination of LL from NMR relaxation measurements was much better for trout than for salmon samples.

## KEYWORDS:

Smoked fish, water holding capacity, liquid loss, low-field NMR

## INTRODUCTION

Cold smoked salmon is a very popular product among consumers. However, during recent years the salmon smoking industry has faced problems such as liquid leakage from the smoked product and as a result, cold smoked salmon has lost favour with the consumer (Schubring, 2006). In order to counteract this, it is important to apply analytical methods which can efficiently assess the quality from raw material to end product and form the basis of recommendations on the most optimal method to produce cold smoked salmon.

Lipid and water make up about 80% of the fish muscle. Depending on the properties of the flesh and the way it is treated, it may gain or lose liquid during processing. This is of economic importance to producers and retailers as the fish is sold by weight. The ability of the flesh to hold liquid and the distribution of water and fat within the flesh are also important to the consumer because they influence both texture and juiciness and thereby contribute to the overall product quality. The ability of the fish muscle to retain its own liquid has traditionally been described by the term water holding capacity (WHC), as most research has been conducted into lean fish species. However, given the increasing production of salmon and trout products, more focus has been directed to the total liquid loss from fatty fish. The liquid loss in these species comprises quite high amounts of lipid, in addition to water. Therefore, the term liquid holding capacity has been introduced, referring to the ability of a given structure to prevent both water and lipid from being released from the structure (Hermansson, 1986). Liquid holding capacity can be expressed in relation to the dry matter content or to the original amount of liquid (Eide, Børresen & Strøm, 1982).

Nowadays, farmed salmon is mainly used in the smoking industry instead of the traditional wild salmon. Along with the increased production of salmon and smoked salmon products, higher losses of liquid during and after smoking of farmed salmon has been observed, and it has been attributed to the high lipid content and the lipid composition in the raw material (Sheenan, O'Connor, Sheehy, Buckley and FitzGerald, 1996). When there is accumulated liquid in vacuum-packaged smoked salmon, it will affect the product appearance negatively (Birkeland, 2004). Farm-reared salmon can have higher lipid content than wild salmon. Farmed Atlantic salmon of market size has lipid content between 6 % and 22 %, with 15-16 % as average in the Norwegian quality cut (Rørå, Kvåle, Mørkøre, Rørvik, Steien & Thomassen, 1998).

Several different methods to determine liquid holding capacity (LHC) and liquid loss (LL) on either intact or minced salmon fish muscle have been used and they can be categorised as either direct or indirect methods.

#### *Direct methods:*

Determination of the ability to hold liquid under influence of an external force by measuring the amount of liquid squeezed out from minced or chopped muscle after exposure to an external force like centrifugation (Jauregui, Regenstein & Baker, 1981; Eide et al. 1982; Hermansson 1986; Gómez-Guillén, Montero, Hurtado & Borderias, 2000). In studies of LHC in salmon or trout, the centrifugation methods by Gómez-Guillén et al. (2000), or that by Hermansson (1986) have been used the most (although with some modification). The two methods are very similarly and use the same parameters for centrifugation (a force of 500 g, a temperature of 10°C, 10 minutes centrifugation and a sample size of 10 or 15 g). In both methods, the released liquid is collected and the amount of lipid and water in the liquid loss is determined.

Another direct method is the filter press method where a filter paper is pressed against the fillet or mince by an external force which can be applied in different ways, e.g. by compression using a Texture Analyser with a flat end probe (Jonsson, Sigurgisladottir, Hafsteinsson & Kristbergsson, 2001; Cardinal, Gunnlaugsdottir, Bjørnevik, Ouisse, Vallet & Leroi, 2004) or simply by the use of a 500 g weight (Løje and Nielsen, submitted). Liquid leakage can also be measured as “free” drip, which refers to the amount of liquid lost without application of an external force (Mørkøre, Hansen, Unander & Einen, 2002).

#### *Indirect methods:*

Water content and water holding capacity can also be measured indirectly by low-field  $^1\text{H}$  nuclear magnetic resonance (NMR) spectroscopy. Low-field  $^1\text{H}$  NMR is an excellent tool for measurement of distribution of water pools in food systems as it is rapid and in principle non-destructive. NMR measures the mobility of protons and is therefore a technique for investigating the total quantity of water and the state of water within the fish muscle. NMR technique has been used for determination of water holding capacity in cod (Jepsen, Pedersen and Engelsen, 1999; Andersen and Jørgensen, 2004) and salmon (Lakshmanan, Parkinson & Piggott, 2007), and the oil and water contents in herring (Nielsen, Hyldig, Nielsen & Nielsen, 2005) and salmon (Bechmann, Pedersen, Nørgaard & Engelsen, 1999; Jepsen et al. 1999). The possibility for using NMR in studying lipid holding capacity exists but has not as yet been explored.

Common for the direct and indirect methods is that they are highly dependent on experimental conditions and must be standardised before use. The methods are among other factors dependent on sample preparation like using pieces of whole muscle or coarse- or fine-minced muscle. And sampling is of outmost importance: biological factors like chemical composition,

biological state and post-mortem processes influence the fish flesh quality and liquid holding (Ofstad, 1995). These factors vary from individual to individual, and also to some extent from head to tail in an individual. In a study by Mørkøre et al. (2002), samples were taken from several places in the filet: neck, dorsal, Norwegian quality cut, tail and belly flap, and the results showed that liquid leakage increased from head to tail of the fresh and frozen fillets, although no significant variation was observed after ice-storage for 11 days at - 20°C. The choice of part of the fillet is also important when preparing samples for NMR-measurements (cf. Andersen & Rinnan, 2002).

Different studies of liquid holding in salmon and trout have used different methods and different experimental conditions making it very difficult to compare the results. The present study examines some current methods with regard to their suitability for determining liquid holding capacity and liquid loss in fatty fish like salmon and trout. Results obtained by a centrifugation method are compared to results obtained by a liquid leakage test applied to the same fish. Low field  $^1\text{H}$  NMR relaxation curves are correlated to values obtained by the centrifugation method and by the liquid leakage test to see if information in the NMR signals can be related to the phenomena measured by these two methods. The study includes raw as well as smoked trout and salmon.

## MATERIALS AND METHODS

### Experimental design

Two experiments, one with salmon and one with rainbow trout, were carried out. In experiment one, four groups of Atlantic salmon (*Salmo salar*) were analyzed. The salmon

came from two farms in Norway with two sizes (3-4 kg and 6-7 kg) of fish from each farm. In experiment two, 32 rainbow trout (*oncorhynchus mykiss*,) from different fish farms in Denmark were used. The trout were divided into two subgroups with 16 fish in each. Subgroup one was ice-stored at 2°C for 5-6 days (short) and subgroup two was ice-stored at 2°C for 12-13 days (long) after slaughtering. Information on the samples is given in Table 1. In both experiments, raw (unprocessed) and smoked fish were analyzed. The smoked samples were chill-stored at 2°C for up to 20 days after smoking. Each code was analysed three times during the storage period.

#### **Preparation of samples (both experiments)**

The fish were filleted by hand and trimmed. The left sides were kept as raw (unprocessed) fillets whereas the right sides were processed into smoked fillets: The fillets were dry-salted (60 g of salt per kg fillet) for five hours for small fish (size 3-4 kg) and eight hours for the large fish (size 6-7 kg) at room temperature. After rinsing, the fillets were chilled and stored overnight at 2°C. The fillets were dried for two hours and smoked for 5½ hours at 26°C in an oven supplied with smoke generated from beechwood, After smoking, the fillets were chilled again and stored overnight at 2°C.

The sampling scheme was similar to that described in Løje, Green-Petersen, Nielsen, Jørgensen and Jensen (2007). Two pieces were cut out from the left fillet side; piece one was in front of the dorsal fin and above the lateral line and piece two was under the dorsal fin and above the lateral line. From piece one, two muscle cubes, each of approximately two grams were cut and used for NMR measurements on intact muscle (named intact samples). The rest of piece one was minced for 2 x 5 s at 5°C in a Knifetec, 1095 Sample Mill (Foss Tecator, Sweden). The mince was used for NMR measurements on minced samples and for

determination of dry matter content and of liquid holding capacity by the centrifugation method. Piece two was used for determination of liquid loss (liquid leakage test). For the smoked fillets, two pieces were cut similar to the treatment of the raw fillets but each further divided into three smaller pieces. Each of the three smaller pieces was vacuum-packed and randomly assigned to one of three storage periods (1, 11 or 19 (trout) or 20 (salmon)) days at 2°C after smoking. After each storage period, analysis was performed in the same way as for raw fish.

## Methods

### *Centrifugation method*

The liquid holding capacity (LHC) was determined on minced fillet from piece one using a modification of the centrifugation method by Eide et al. (1982). The samples (2 g) were weighed, placed in plastic tubes with a special filter bottom (pore size 0.1 mm), centrifuged (1500 g, 10°C, 5 min) and weighed again. The liquid holding capacity was calculated in two ways: as the amount of liquid left in the mince after centrifugation relative to either the original amount of liquid (LHC<sub>1</sub>) or the dry matter content (LHC<sub>2</sub>). The measurement of the liquid holding capacity was performed in quadruplicate.

### *Liquid leakage test*

The liquid leakage test by Mørkøre et al. (2002) was used with some modification to determine the liquid loss (LL). A slice of untrimmed muscle (about 15 g) from piece two was placed on a pad (7.5 x 12.5 cm) from Cryovac Lit Loc 800, wrapped in aluminium foil and kept at room temperature for 20 h. A piece of nylon cloth was placed between the muscle and the pad to avoid the muscle from sticking to the pad. After removal of the muscle piece, the pad was dried at 105°C for 60 min in order to evaporate all the water. The weight of the pad



was recorded initially ( $w_a$ ), after 20 hours ( $w_b$ ) and again after drying ( $w_c$ ). The liquid loss in % was calculated as  $100 (w_b - w_a)/m$ , where  $m$  is the weight of the muscle sample. In experiment two, the sample size was reduced to about 10 g due to the fish size, a Whatman filter paper GF/A 125 mm was used instead of a pad, and the filter paper was put in a closed Petri dish instead of wrapped in aluminium foil. Otherwise, the same procedure was followed as in experiment one. Four determinations were made for each sample.

#### *Nuclear Magnetic Resonance (NMR) measurements*

Low-field NMR relaxation measurements were performed by a Maran 23 MHz Pulsed NMR analyser (Resonance Instruments, UK) as described by Jensen, Guldager and Jørgensen (2002). Transverse relaxation was measured at 8°C using the CPMG pulse sequence (Carr and Purcell, 1954, Meiboom and Gill, 1958) with an inter-pulse spacing,  $\tau$ , of 200  $\mu$ s and sampled each  $4\tau$  (even echoes) to a total of 512 data points.

#### *Salt content*

The salt content was determined according to AOAC methods (AOAC method 976.18 in combination with AOAC method 937.07 and AOAC method 971.27 2000).

#### *Water content*

The water content was calculated from 2 g mince dried overnight at 105°C.

#### *Lipid content*

The lipid content was determined by a modified version of the Bligh & Dyer extraction method (Bligh and Dyer, 1959).

#### **Data analysis**

1  
2 Statistical tests (t-test, correlation and one-way ANOVA) were made using Prism® version 4  
3 (GraphPad Software, San Diego, CA, USA). The NMR relaxation curves were divided by  
4 sample weight (approx 2 g) and the number of data points reduced by a factor of four (taking  
5 the means of four neighbouring points). A principal component analysis (PCA) of the NMR  
6 relaxation curves revealed one outlying measurement on the intact salmon samples and three  
7 on the minced salmon samples. These measurements were excluded from further analysis.  
8 Partial least squares regression (PLSR) was used to calculate regression models between the  
9 reference methods (centrifugation or liquid leakage test) and the NMR relaxation curves. The  
10 NMR relaxation curves (average over the two determinations) were used as  $x$ -variables and  
11 LHC or LL as  $y$ -variable in the PLSR models. Four calibration models were made (intact  
12 salmon, intact trout, minced salmon, minced trout). The raw and smoked samples were  
13 included in the same data set. Cross-validation with 10 % of the samples, randomly selected,  
14 in each segment was used. Variables were mean-centred but not weighted. The PLSR models  
15 were evaluated by the correlation between the measured and the predicted values and by the  
16 root mean squared error of prediction (RMSEP).

17  
18 PCA was performed on a data matrix with LL, LHC (both  $LHC_1$  and  $LHC_2$ ), salt content and  
19 water contents as variables. The single variables were mean-centred and weighted with  $1/SD$ ,  
20 where  $SD$  referred to the standard deviation for each variable across samples. PCA models  
21 were calculated for smoked samples. Cross validation was performed as described for the  
22 PLSR models above. All multivariate data analysis was performed with The Unscrambler®  
23 version 9.1 (CAMO, Oslo, Norway).

## 24 25 **RESULTS AND DISCUSSIONS**

## Liquid holding capacity (LHC)

The principle in all centrifugation methods is that a centrifugal force is used as an external force. Other parameters may differ from one method to another and these parameters can be sample preparation (chopped or minced sample), time and speed of centrifugation, temperature during centrifugation, size of centrifuge tubes, etc. The location for taking muscle samples has to be standardized as the properties of the fillet vary somewhat from the head to the tail region (Love, 1988). Although the whole sample/fish as chopped fillets or mince may be used to avoid this. Results obtained by different laboratories' centrifugation methods usually cannot be compared as at least one of the above-mentioned parameters often differ.

The liquid holding capacity (LHC) was here determined using the centrifugation method by Eide et al. (1982). In other studies of LHC in salmon or trout, mainly two other centrifugation methods have been used (with some modification): either the one by Gómez-Guillén et al. (2000), or that by Hermansson (1986). The three methods differ according to how the results are calculated and according to centrifugation settings.

In the present study, the raw samples (both salmon and trout) had higher LHC relative to dry matter content (LHC<sub>2</sub>) than had the smoked samples ( $p < 0.001$ ). For smoked salmon samples, a decrease in LHC (both LHC<sub>1</sub> and LHC<sub>2</sub>) was observed between day 1 and day 20 ( $p < 0.05$ ) and between day 11 and day 20 ( $p < 0.01$ ) (Løje, Jensen, Hyldig, Nielsen and Nielsen, submitted). This was also seen for smoked trout samples, as a decrease in LHC (both LHC<sub>1</sub> and LHC<sub>2</sub>) was observed between day 1 and day 19 ( $p < 0.001$ ) and between day 11 and day 19 (LHC<sub>1</sub>:  $p < 0.001$ ; LHC<sub>2</sub>  $p < 0.05$ ). (Table 2). In other studies, lower LHC values (indicated by higher liquid loss) have been observed for smoked salmon compared to raw (Birkeland,

Rørå, Skåra & Bjerkeng, 2004). However, in another study, Rørå, Regost & Lampe (2003) reported a higher LHC (indicated by lower liquid loss) in smoked salmon than in raw. The levels of liquid loss determined in Birkeland et al (2004) by the centrifugation method were in range of 2.5 to 4.7 % for raw salmon and between 3.3 and 5.9 % for smoked salmon. In the present study, the liquid loss during centrifugation was between 2.0 and 13.4 % (average 5 %) for raw salmon and between 0.7 and 19.3% (average 7.4 %) for smoked salmon. LHC (according to original liquid amount) was higher for small salmon samples than for smoked samples (seen for each day of storage) . Chill storage of the raw trout before processing had no effect on the LHC (both LHC<sub>1</sub> and LHC<sub>2</sub>).

#### *Liquid loss (LL)*

The liquid leakage test by Mørkøre et al. (2002) gives an indication of the ability of the material to withhold water and lipid without severely changing the microstructure of the sample. LL thus is more closely relevant to the appearance of the product than is LHC. LL from salmon was higher than from trout. For both species, LL was higher for the raw fish than for the newly smoked fish (Table 3). During chill storage at 2°C of the smoked salmon samples, a increase in liquid loss was observed between day 1 and day 20 ( $p < 0.001$ ) and between day 11 and day 20 after smoking ( $p < 0.001$ ). High LL of raw compared to smoked fish was also found by Rørå et al. (2003), the raw fish losing about 3.5 % and the smoked fish about 2 % of the liquid. After 15 days of storage however, LL of the smoked fillet was at the same level as seen for the raw fillet. For smoked salmon, an increased in the LL was observed during chill storage of the smoked product (Table 3). This was also reported by Rørå et al. (2003), who found an LL after 15 days of storage of 2.8 % (storage temperature 4 °C) and 6.3 % (storage temperature 14 °C). A similar development in LL during chill storage was not observed for the smoked trout samples.

The size of the fish had some influence on the LL as large smoked fish lost significantly ( $p < 0.001$ ) more liquid than did the smaller smoked fish in raw and newly smoked fish. After 11 days of storage this difference had gone.

In the present study, the method by Mørkøre et al. (2002) was used with some modifications and changes were made from experiment one to experiment two. A high standard deviation (four determinations) for each sample was seen in experiment one. Therefore, filter paper was used in experiment two, and the samples were stored in a Petri dish instead of being wrapped in aluminium foil. By using a Petri dish, the filter paper with the fish sample was kept under more stable conditions, and the risk of liquid escaping, e.g. by evaporation, minimised. These changes seemed to give more reliable results as the measurements in experiment 2 had lower standard deviations than those in experiment 1.

#### *Correlation between the centrifugation method and the liquid leakage test*

The liquid leakage test and the centrifugation method measure different properties (liquid loss from intact muscle with external force applied to the sample surface and from minced muscle with external force applied to the full sample). Accordingly, the liquid leakage test and the centrifugation method were poorly (although significantly) correlated for smoked salmon ( $r = -0.28$ ,  $p < 0.05$ ) and for smoked trout ( $LHC_1$ ,  $r = -0.25$ ,  $p < 0.05$ ). For raw trout samples, LL was negatively correlated to  $LHC_1$  ( $r = -0.41$ ,  $p < 0.05$ ). For raw salmon samples, the correlation coefficients between LL and LHC were insignificant.

Rørå et al. (2003) compared the centrifugation method by Gómez-Guillén et al. (2000) with the liquid leakage method by Mørkøre et al. (2002) on smoked salmon. They found a high correlation for liquid and lipid holding capacity, but no correlation for water holding capacity.

Mørkøre (2001) found a high correlation between liquid loss measured by the liquid leakage method and by the centrifugation method for raw salmon. Both Mørkøre (2001) and Rørå et al. (2003) used slices of muscle samples for the centrifugation method, whereas mince was used for the centrifugation method in the present study. Rørå et al. (2003) reported a lower liquid loss when using the centrifugation method than the liquid leakage test. In the present study, the opposite was observed as higher liquid loss was obtained by the centrifugation method compared with the liquid leakage method. Especially for the large salmon, a big difference was seen between the two methods. The difference in liquid loss determined by the two methods seems to be reasonable as an external force may press out more liquid than a 'passive' method without any external force. Furthermore, liquid may escape mince more easily than intact tissue as mincing partly destroys the muscle cells.

A PCA was made of a data set containing LHC (both  $LHC_1$  and  $LHC_2$ ), LL, and salt and water contents of smoked salmon samples. The relationships between the variables are shown in Figure 1 as the so-called correlation loadings. Principle component 1 described mainly the variation in LHC ( $LHC_1$  and  $LHC_2$ ) and water content, while Principal component 2 described mainly the variation in LL. The changes in LL were almost independent of changes in LHC and water contents. Similar PCA results were obtained for smoked trout samples.

#### *Relating NMR curves to liquid holding capacity and liquid loss*

The NMR relaxation curves were used to predict LHC and LL for minced and intact samples, respectively (Table 4 and 5), by partial least squares regression. Prediction of LHC (both  $LHC_1$  and  $LHC_2$ ) from the NMR relaxation curves worked well for minced salmon and minced trout (Table 4). A tendency of non-linearity was observed when predicting  $LHC_1$  though. The correlation coefficients were close to that reported by Andersen and Jørgensen

(2004) for cod. With intact samples, good relation was found between the NMR relaxation curves and  $LHC_1$  or  $LHC_2$  for salmon and  $LHC_2$ , but not  $LHC_1$ , for trout (Table 5).

The prediction of LL from NMR relaxation curves was much better for trout than for salmon. The salmon had the highest lipid content and there appears to be a relation between higher lipid content and more lipid in the liquid loss: the lipid loss with time from salmon was higher relative to the water loss than was the case from trout (data not shown). NMR measures the quantity and mobility of protons and is therefore a technique for investigating the total amount of water and the state of water within the fish muscle. This may be one of the reasons why the prediction of LL was better for trout than for salmon. In addition, changes in details of the LL method had been made from experiment 1 to experiment 2 to optimize the method, which also may have influenced the results.

Models for prediction of LL from the NMR data were made for raw and smoked samples separately. These models were inferior to those including both raw and smoked samples indicating that LL unlike LHC is not closely related to the water distribution in the fish muscle but governed by other properties of the fillet. The information in the NMR signals used by the calibration with the full data set is rather about water content which like LL differs between the groups of raw and smoked fish.

#### *Comparing advantages and disadvantages of the methods*

The liquid leakage test was able to detect differences in liquid loss between raw and smoked sample. Changes in liquid loss due to chill storage of the smoked fish were seen for salmon, the largest change occurring between 11 and 20 days. A similar change with storage time was not observed for smoked trout. The lipid content was considerably higher in the salmon than in the trout which might at least to some extent explain this difference between the two

species. The centrifugation method was able to detect changes due to sample type (raw and smoked) and also due to chill storage of the smoked product from both fish species. The method to determine LHC does, however, make use of laboratory facilities and therefore is less suitable in a production environment. The liquid leakage test may be conducted without any advanced equipment and furthermore is mimicking the drip loss method used in the industry. The liquid leakage test and the centrifugation method both require standardized conditions, as they are dependent on numerous experimental conditions during measurement. One is temperature, but a lot of others influence the results, and further investigations is needed before the liquid leakage test can be general recommended.

Mørkøre (2001) found that an untrimmed muscle slice weighing about 15 g was an appropriate sample size as larger samples occasionally lost so much liquid that the pad became overloaded and the liquid escaped. However it is very important to be able to make reproducibly cutting of these 15 g from the fillet. The effect of variations in sample size needs to be explored. If the piece is thick, its surface area will be smaller and the pressure therefore larger compared to a thin piece. And even if the cutting area is well defined, there will be a difference between small fish and large fish. The optimal method is to use a template to cut reproducible samples for each determination. Leaving the pad and muscle wrapped in aluminium foil for 20 hours at room temperature was suitable for detecting differences in LL between treatments (Mørkøre, 2001). However, this statement needs to be tested for other fatty fish and for large fish. The liquid leakage test was used by Mørkøre et al. (2002) on salmon with a weight of 4 kg. In the present study, some of the fish weighted 6-7 kg, and the results show that these fish lost more liquid than did the smaller fish. It is of importance to be confident that the pad or filter paper absorbs all the released liquid. Another important aspect which has to be considered when using the liquid leakage test on smoked fish is the smoke hue. The smoke hue will partly hinder liquid loss from the sample. If the smoke hue in some



way is damaged in one fish compared to another otherwise similar one, there is a risk of an artificially high LL. The smoke hue also makes it difficult to compare results from raw fish with those from smoked fish. Both in the present study and in the study by Rørå et al. (2003) higher liquid losses were obtained for raw compared to newly smoked samples. The effect of the smoked hue on the results should be further studied.

The liquid leakage method has only been used in a few studies, and in these studies the method has been used either to detect the effect of different oil source in the feed (Rørå et al., 2003) or to see effects of freezing (Mørkøre et al., 2002) on small salmon (weight 1- 4 kg). The liquid leakage method has its potential, but the above mentioned parameters need to be tested. One should also have in mind that the centrifugation method and the liquid leakage test measure different properties, so substitution of one with the other is not always an option.

Low field NMR relaxation curves were used to get some insight into the differences between the properties assessed by the centrifugation method and the liquid leakage method. In addition, NMR itself can be used as a rapid and non-destructive method for determination of water-holding capacity in muscle tissue (Jepsen et al., 1999; Andersen and Jørgensen, 2004; Lakshmanan et al., 2007). In the present study it was possible to obtain good predication of LHC from NMR relaxation curves measured on minced salmon samples with LHC values between 84-99% (LHC<sub>1</sub>). The applicability of NMR relaxation curves to samples with lower LHC values has not yet been tested.

## CONCLUSION

The ability of the fish muscle to hold liquid is a complex property influenced by many factors. The various methods used for assessing this property focus on different aspects making

comparison of results difficult. The present paper demonstrates that two of the most popular approaches, measuring the liquid holding capacity by centrifugation of minced muscle and measuring the liquid loss by the liquid leakage test, provide supplementary rather than redundant information. Thus, it is recommended to use both types of methods where applicable in order to get as detailed picture as possible of this important quality parameter.

## ACKNOWLEDGEMENT

This work was financially supported by grants from The Danish Ministry of Food, Agriculture and Fisheries. The authors are very thankful to Dr. Kristina N. Jensen and Ms. Sally Clink for valuable discussions during preparation of the manuscript, to Mr. Carsten Østerberg for assistance with salting and smoking and determination of liquid holding capacity, and to Mr. Ulrik Cold for technical assistance with the NMR measurements and to Ms. Karin Reimers for the liquid leakage test..

## REFERENCES

- Andersen, C. M. & Jørgensen, B. M. (2004). On the relation between water pools and water holding capacity in cod muscle. *Journal of Aquatic Food Product Technology*, 13, 13-23.
- Andersen, C. M. & Rinnan, Å (2002). Distribution of water in fresh cod. *Lebensmittel-Wissenschaft und Technologie*, 35, 687-696.
- AOAC. (2000). Method no. 976.18 "Salt (Chlorine as Sodium Chloride) in Seafood. Potentiometric Method". Official Methods of Analysis, AOAC, Arlington, VA.

1 AOAC. (2000). Method no. 937.07 “Fish and marine products. Treatment and preparation of  
2 sample procedure”. Official Methods of Analysis, AOAC, Arlington, VA.

3  
4 AOAC. (2000). Method no. 971.27 “Sodium chloride in canned vegetables. Potentiometric  
5 method”. Official Methods of Analysis, AOAC, Arlington, VA.

6  
7 Bechmann, I. E., Pedersen, H. T., Nørgaard, L., & Engelsen, S. B. (1999). Comparative  
8 chemometric analysis of transverse low-field  $^1\text{H}$  NMR relaxation data, In Advances in  
9 Magnetic Resonance in Food Science, pp 217-225 Belton PS, Hills BP, and Webb GA, The  
10 Royal Society of Chemistry, Cambridge

11  
12 Birkeland, S. (2004). Cold-smoking of Atlantic salmon (*Salmo salar*) – Influence of  
13 processing conditions and raw material characteristics on quality and yield. Doctor Thesis,  
14 Department of Animal and Aquacultural Sciences, Agricultural University of Norway.

15  
16 Birkeland, S., Rørå, A. M. B., Skåra, T. & Bjerkeng, B. (2004). Effects of cold smoking  
17 procedures and raw material characteristics on product yield and quality parameters of cold  
18 smoked Atlantic salmon (*Salmo salar* L) fillets. *Food Research International*, 37, 273-286.

19  
20 Bligh EG and Dyer WJ, (1959). A rapid method of total lipid extraction and purification. *Can*  
21 *J Biochem Physiol*, 37: 911-917.

22  
23 Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J. L., & Leroi, F.  
24 (2004). Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European  
25 market and relationships with chemical. physical and microbiological measurements. *Food*  
26 *Research International*, 37, 181-193.

- 1
- 2 Carr, H. Y. & Purcell, E. M. (1954). Effect of diffusion free precession in nuclear magnetic  
3 resonance experiments. *Physical Review* 94, 630-638.
- 4
- 5 Eide, O., Børresen, T. & Strøm, O. (1982). Minced fish production from capelin (Mallotus  
6 villosus). A new method for gutting, skinning and removal of fat from fatty species. *Journal*  
7 *of Food Science*, 47, 347-349, 354.
- 8
- 9 Gómez-Guillén, M.C., Montero, P., Hurtado, O. & Borderías, A. J. (2000). Biological  
10 characteristics affect the quality of farmed Atlantic salmon and smoked muscle. *Journal of*  
11 *Food Science*, 65, 53-60.
- 12
- 13 Hermansson, A. M. (1986). Water- and fat holding. In Mitchell J. R. & Ledward D. A.  
14 Functional properties of food macromolecules (pp 273-314). London: Elsevier Ltd.
- 15
- 16 Jauregui, C. A., Regenstein, J. M. & Baker, R. C. (1981). A simple centrifugal method for  
17 measuring expressible moisture. a water-binding property of muscle food. *Journal of Food*  
18 *Science*, 46, 1271-1273.
- 19
- 20 Jensen, K. N., Guldager, H. S. & Jørgensen, B. M. (2002). Three-way modelling of NMR  
21 relaxation profiles from thawed cod muscle. *Journal of Aquatic Food Product Technology*,  
22 11, 201-214.
- 23
- 24 Jepsen, S. M., Pedersen, H. T & Engelsen, S. B. (1999). Application of chemometrics to low-  
25 field relaxation data of intact fish flesh. *Journal of the Science of Food and Agriculture*, 73,  
26 1793-1802.

- Jonsson, A., Sigurgisladdottir, S., Hafsteinsson, H. & Kristbergsson, K. (2001). Textural properties of raw Atlantic salmon (*Salmo salar*) fillets measured by different methods in comparison to expressible moisture. *Aquaculture Nutrition*. 7, 81-89.
- Lakshmanan, R., Parkinson, J.A. & Piggott, J.R. (2007). High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (*Salmo salar*). *Lebensmittel-Wissenschaft und –Technologie*. 40, 544-551
- Love, R. M. (1988). The food fishes: Their intrinsic variation and practical implication (pp 3-42).. Farrand Press, London
- Løje, H., Green-Petersen, D., Nielsen, J., Jørgensen, B. M. & Jensen, K. N. (2007). Water distribution in smoked salmon. *Journal of the Science of Food and Agriculture*, 87, 212-217
- Meiboom, S. & Gill, D. (1958). Modified spin-echo method for measuring nuclear times. *The Review of Scientific Instruments* 29, 688-691.
- Mørkøre, T. (2001). Texture, fat content and product yield of salmonids. Dr. Thesis  
Department of Animal and Aquacultural Sciences, Agricultural University of Norway.
- Mørkøre, T., Hansen, A. Å., Unander, E. & Einen, O. (2002). Composition, liquid leakage and mechanical properties of farmed rainbow trout: Variation between fillets sections and the impact of ice and frozen storage. *Journal of Food Science*, 67, 1933-1938.

- 1 Nielsen, D., Hyldig, G., Nielsen, H. H. & Nielsen, J., (2005) Lipid content in herring (*Clupea*  
2 *harengus* L.) – Influence of biological factors and comparison of different methods of  
3 analyses: solvent extraction, Fatmeter, NIR and NMR. *Food Science and Technology* 38( 5),  
4 537-548  
5
- 6 Ofstad, R. (1995) Microstructure and liquid holding capacity in cod (*Gadus morhua* L.) and  
7 salmon (*Salmo salar*) muscle; effects of heating. Dr. Scient. Thesis, Insititute of Medical  
8 Biology, University of Tromsø  
9
- 10 Rørå, A.M.B., Kvåle, A., Mørkøre, T., Rørvik, K-A., Steien, S.H. & Thomassen M.S. (1998).  
11 Process yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*) in relation  
12 to raw material characteristics. *Food Research International*, 31(8), 601-609  
13
- 14 Rørå, A. M. B., Regost, C. & Lampe, J. (2003). Liquid holding capacity. texture and fatty  
15 acid profile of smoked fillets of Atlantic salmon fed diets containing fish oil or soybean oil.  
16 *Food Research International*. 36, 231-239  
17
- 18 Schubring, R. (2006). Thermal stability, texture, liquid holding capacity and colour of smoked  
19 salmon on retail level. *Thermochimica Acta*, 445, 168-178  
20
- 21 Sheehan, E. M., O'Connor, T. P. O., Sheehy, P. J. A., Buckley, D. J. and FitzGerald, R.  
22 (1996). Effect of dietary fat intake on the quality of raw and smoked salmon. *Irish Journal of*  
23 *Agricultural and Food Research*, 35 (1): 37-42

Table 1. Information about the samples

	Experiment 1	Experiment 2
Sample type	Atlantic salmon ( <i>Salmo salar</i> )	Rainbow trout ( <i>oncorhynchus mykiss</i> ,)
Harvest	May 2004	November 2004
Farmed in	Norway	Denmark
Size	3-4 kg and 6-7 kg	2 to 3 kg
Groups	Four	Two

Table 2. Liquid holding capacity

			Storage time after smoking			
	Code		Raw	Day 1	Day 11	Day 19/20
Exp. 1 (salmon)	Farm 1 small	LHC <sub>1</sub>	95.6 ± 2.1	94 ± 2.6	94.6 ± 2.6	92.7 ± 3.7
	(N = 7)	LHC <sub>2</sub>	3.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	2.2 ± 0.2
	Farm 2 small	LHC <sub>1</sub>	97.6 ± 0.6	95.9 ± 2.7	96.8 ± 3	93.7 ± 4.8
	(N = 6)	LHC <sub>2</sub>	3.7 ± 0.1	2.5 ± 0.2	2.6 ± 0.1	2.3 ± 0.4
	Farm 1 large	LHC <sub>1</sub>	87.3 ± 1	85.6 ± 1.9	82.7 ± 0.4	80.8 ± 0.1
	(N = 2)	LHC <sub>2</sub>	3.3 ± 0.4	2.5 ± 0.5	2.4 ± 0.4	2.0 ± 0.2
	Farm 2 large	LHC <sub>1</sub>	93.6 ± 5.5	89.0 ± 3.8	91.9 ± 2.2	89.9 ± 4.3
	(N = 3)	LHC <sub>2</sub>	3.4 ± 0.3	2.1 ± 0.2	2.4 ± 0.1	2.3 ± 0.1
Exp. 2 (trout)	Short	LHC <sub>1</sub>	96.2 ± 1.5	96.3 ± 2.7	96.3 ± 3.8	93 ± 5.8
	(N = 16)	LHC <sub>2</sub>	3.4 ± 0.2	2.3 ± 0.1	2.3 ± 0.2	2.1 ± 0.4
	Long	LHC <sub>1</sub>	96.7 ± 0.2	97.7 ± 1.6	96 ± 2.8	94.6 ± 2.7
	(N = 16)	LHC <sub>2</sub>	3.2 ± 0.1	2.2 ± 0.2	2.1 ± 0.3	1.9 ± 0.3

Values are means ± standard deviation

N is number of samples

LHC<sub>1</sub> = the amount of liquid left in the mince after centrifugation relative to the original amount of liquid

LHC<sub>2</sub> = the amount of liquid left in the mince after centrifugation relative to the dry matter

content



Table 3. Liquid loss (%)

		Storage time after smoking			
	Code	Raw	Day 1	Day 11	Day 19 /20
Experiment 1	Farm 1 small	$2.8 \pm 0.5$	$1.0 \pm 0.3$	$1.6 \pm 0.4$	$3.0 \pm 0.9$
	(N = 7)				
	Farm 2 small	$2.8 \pm 0.5$	$1.0 \pm 0.2$	$1.5 \pm 0.4$	$1.7 \pm 0.6$
	(N = 6)				
	Farm 1 large	$3.7 \pm 0.2$	$1.9 \pm 0.3$	$1.5 \pm 0.1$	$2.7 \pm 0.4$
Experiment 2	(N = 2)				
	Farm 2 large	$4.3 \pm 0.2$	$1.7 \pm 0.1$	$1.9 \pm 0.5$	$3.6 \pm 1.1$
	(N = 3)				
	Short	$2.0 \pm 0.3$	$0.6 \pm 0.2$	$0.7 \pm 0.2$	$0.8 \pm 0.2$
	(N = 16)				
	Long	$2.2 \pm 0.6$	$0.7 \pm 0.2$	$0.6 \pm 0.2$	$0.6 \pm 0.2$
	(N = 16)				

Values are means  $\pm$  standard deviation

N is number of samples

Table 4. Results from PLSR models for predicting e.g. liquid loss (LL) and liquid holding capacity (LHC) (Y) from NMR relaxation curves (X) obtained for minced samples.

	Exp. 1			Exp. 2		
	LHC <sub>1</sub>	LHC <sub>2</sub>	LL	LHC <sub>1</sub>	LHC <sub>2</sub>	LL
No. samples	70	70	70	128	128	127
no. components <sup>a</sup>	2	2	3	4	4	4
Explained Y- variance <sup>a</sup>	71	78	33	64	86	82
RMSEP <sup>b</sup>	2.7	0.3	0.9	2.1	0,2	0.3
Correlation <sup>c</sup>	0.83	0.88	0.57	0.79	0.93	0.90

a: Optimal number of PLS-components in the regression model and % variation according to liquid loss or LHC explained by the validated calibration model estimated by cross validation

b: Prediction error. Root mean square error of prediction

c: Model fit. Correlation between measured and predicted liquid loss or LHC values

Table 5. Results from PLSR models for predicting e.g. liquid loss (LL) and liquid holding capacity (LHC) (Y) from NMR relaxation curves (X) obtained for intact samples.

	Exp. 1			Exp. 2		
	LHC <sub>1</sub>	LHC <sub>2</sub>	LL (%)	LHC <sub>1</sub>	LHC <sub>2</sub>	LL (%)
No. samples	71	70	70	m <sup>d</sup>	63	63
no. components <sup>a</sup>	2	3	3		4	4
Explained y- variance <sup>a</sup>	70	53	22		74	76
RMSEP <sup>b</sup>	2.7	0.4	0.9		0.3	0.3
Correlation <sup>c</sup>	0.83	0.75	0.46		0.85	0.87

a: Optimal number of PLS-components in the regression model and % variation according to liquid loss or LHC explained by the validated calibration model estimated by cross validation

b: Prediction error. Root mean square error of prediction

c: Model fit. Correlation between measured and predicted liquid loss or LHC values

d: no model calculated

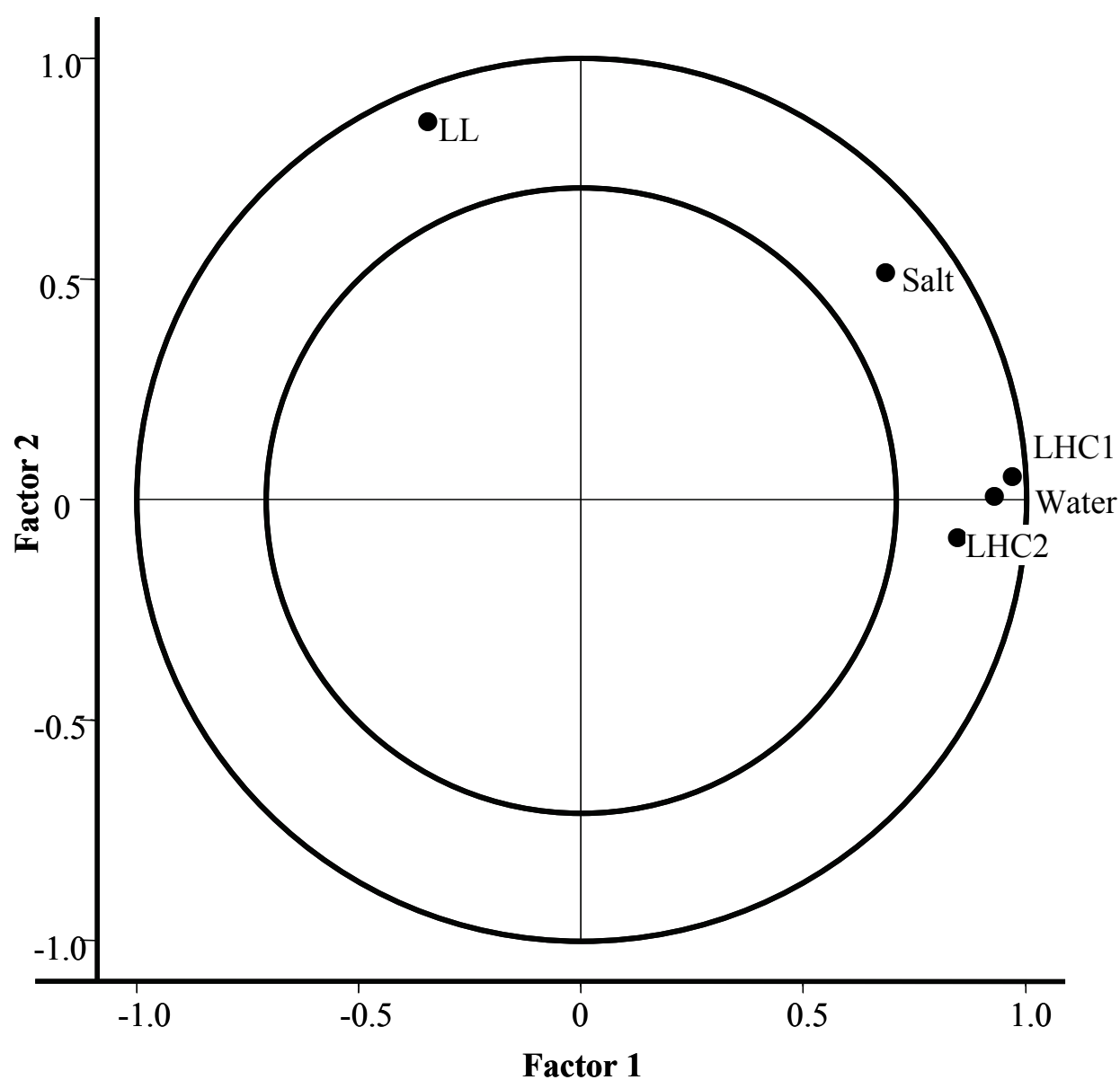


Figure 1. Loading plot from a Principal Component Analysis (PCA) showing the relations between liquid loss (LL), LHC (LHC<sub>1</sub> and LHC<sub>2</sub>), salt and water contents (%) for smoked salmon samples.